The Characterisation of Circulating Biomarkers and Body Composition Before and After Cardiac Resynchronisation Therapy in Patients with Chronic Heart Failure and their Role in Predicting Response

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DEDICATION

I wish to dedicate my thesis to my wonderful wife Dr Anna McAloon, our beautiful children Isabelle and William.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

TABLE OF APPENDICES

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DECLARATION

This thesis is submitted to the University of Warwick in support of my application for the degree of Doctor of Philosophy. It has been composed by myself and has not been submitted in any previous application for any degree .

I am aware of the University regulations governing plagiarism and I declare that this thesis represents my own work except where I have stated otherwise.

The work presented (including data generated and data analysis) was carried out by the author.

All sources are credited by means of referencing.

Dr Christopher McAloon MBChB, MRCP, PGCME

ABBREVIATIONS

4PL	Four-Parametic Logistic Regression
6MWD	6-Minute Walk Distance
6MWT	Six Minute Walk Test
ACC	American College of Cardiology
ACEi	Angiotensin Cardioverting Enzyme Inhibitor
AF	Atrial Fibrillation
AHA	American Heart Association
ANOVA	Analysis of variance
AUC	Area Under Curve
BBB	Bundle Branch Block
BIA	Bioelectrical Impedence Analysis
BMI	Body Mass Index
BNP	Brain Naturetic Peptide
BSA	Body Surface Area
BSA	Bovine Saline Albumin
CC	Cardiac Cachexia
CCS	Clinical Composite Score
cDNA	complementary DNA
CHF	Chronic Heart Failure
CI	Confidence Interval
CITP	Carboxy-Terminal Telopeptide of Type I Collagen
CKD	Chronic Kidney Disease
CMR	Cardiac Magnetic Resonace
CRT	Cardiac Resynchronisation Therapy
CRT-d	CRT-defibrillator
CRT-p	CRT-pacemaker
CS	Coronary Sinus
CTx	Collagen I C-terminal telopeptides
CV	Cardiovascular
CV	Coefficent of Variability
DNA	Deoxyribonucleic Acid
dNTP	Deoxynucleoside Triphosphate
dTTP	Deoxythymidine triphosphate
ECG	Electrocardiogram
ECM	Extracellular Matrix

ELISA	Enzyme-Linked Immunosorbent Assays
ESC	European Society of Cardiology
GDF-15	Growth Differentiation Factor 15
GFR	Glomerular Filtration Rate
HF	Heart Failure
HMRU	Human Metabolic Research Unit
HRS	Heart Rhythm Society
hs-CRP	high sensitive-C reactive protein
hs-TnT	High Sensitvity Troponin T
ICD	Implantable Cardioverter Defirillatior
ICTP	Cardoxyterminal Telopeptide of Type I Collagen
IL-18	Interlukin-18
IL-6	Interlukin-6
IQR	Interquartile Range
IVCD	Interventricular Conduction Disturbance
IVMD	Intraventricular Mechanical Delay
LBBB	Left Bundle Branch Block
LOCF	Last observation carried forward
LV	Left Ventricle
LVAD	Left Ventricular Assist Device
LVEDD	LV End-Diastolic Diameter
LVEDV	Left Ventricular End Diastolic Volume
LVEF	Left Ventricular Ejection Fraction
LVESV	Left Ventricular End Systolic Volume
LVESVI	LVESV indexed by body surface area
MACE	Major Adverse Cardiovascular Events
MAR	Missing at Random
MCAR	Missing Completely at Random
MI	Myocardial Infarction
miRNA	Micro Ribonucleic Acids
MLHFQ	Minnesota Living with Heart Failure Questionnaire
MMP	Matrix Metalloproteinases
MNAR	Missing Not at Random
MR	Mitral Regurgitation
MRA	Mineralcorticoid Receptor Antagonist

MR-proADM	pro-adrenomedullin
MR-proANP	midregional proANP
NHS	National Health Service
NICE	National Institute of Health and Care Excellence
NIVCD	Non-Specific intraventricular Conduction Delay
NYHA	New York Heart Association
OMT	Optimal Medical Therapy
OR	Odds Ratio
PBS	Phosphate Buffered Saline
PCI	Percutanous Coronary Intervention
PCR	Polymerase Chain Reaction
PICP	Carboxy-Terminal Propeptide of Procollagen Type I
PNIIIP	Aminoterminal Propeptides of Type III Collagen
PNIP	Aminoterminal Propeptides of Type I Collagen
PPP	Platelet Poor Plasma
PV	Peripheral Venous
QALY	Quality Adjusted Life Year
QoL	Quality of Life
qPCR	quantatative Polymerase Chain Reaction
Q-Q Plots	Quartile-Quartile Plots
RA	Right Atrium
RAA	Right Atrial Appendage
RBBB	Right Bundle Branch Block
REC	Regional Ethics Committee
RNA	Ribonucleiconic Acid
ROC	Receiver Operating Characteristics
RQ	Relative Quantification
RV	Right Ventricle
RVA	Right Ventricular Apex
RVS	Right Ventricular Septum
SD	Standard deviation
SE	Standard Error
sST2	Soluble concentration of ST2
TGF-ß1	Transforming Growth Factor-ß1
TIMP	Tissue Inhibitors of Metalloproteinases

TNC	Tenascin-C
TPA	Tripropylamine
UHCW	University Hospital Coventry and Warwickshire
VE/VCO_2	Minute Ventilation/Minute Volume CO_2 production
VF	Ventricular Fibrillation
VO_2	Volume of Oxygen
$\dot{V}O_2max$	Oxygen consumption at peak exercise
VT	Ventricular Tachycardia

ABSTRACT

Heart failure is a common condition which carries a high mortality and morbidity. Despite improved medical therapy the outcomes for heart failure with a reduced ejection fraction remain poor. Cardiac resynchronisation therapy has revolutioned the treatment of patients with heart failure with a reduced ejection fraction and dyssynchrony, refactory to medical therapy, improving morbidity and mortality. Unfortunately a significant minority fail to respond to this expensive therapy, which is challenging for both the patient and society.

Over the last 15 years research has focused on attempting to predict non-response. Evidence suggests wider QRS duration and bundle branch morphology on the resting electrocardiograph are the most important predictors of response and outcome following implantation of a cardiac resynchronisation device. However, the non-response rate remains unchanged despite extensive research.

Molecular systems have been shown to alter with the development and progression of heart failure. Many of these systems are now utilised in the diagnosis and prognostication of heart failure. Cardiac resynctonisation therapy device implantation has been shown to alter these dysregulated molecular systems. Specific circulating biomarkers reflect these respective systems. Cardiac extracellular matrix is a dynamic support structure that has altered turnover in heart failure and is affected when cardiac resynchonisation devices are implantted. Micro ribonucleic acids have been observed recently to be important in molecular systems regulation and dysregulation has been observed in heart failure.

Futhermore altered expression following cardiac resynchronisation therapy device implantation has been reported. The evidence suggests circulating biomarkers for these systems have the potential to predict response. Our prospective study examined specific biomarkers that the literature suggests has the potential to predict response, but the evidence is currently inconclusive. Moreover we utilised other important patient variables known to be predictors from the wider literature and our own retrospective cohort analysis of response to test alongside specific circulating biomarkers. We offer an informed pilot study to test important circulating biomarkers for their clinical ultility to predict heart failure patient's ability to respond to cardiac resynchronisation therapy.

Chapter One

INTRODUCTION AND BACKGROUND

1.1 HEART FAILURE

Chronic Heart Failure (CHF) is a common condition defined as an abnormality in cardiac structure and function that leads to the inability of the heart to deliver adequate levels of oxygen to match metabolic demand of the tissues.[2] Patients suffer from a plethora of symptoms including breathlessness, ankle oedema and fatigue.[2] Heart Failure (HF) affects 800,000 people in the UK alone.[2-4] The estimated lifetime risk of developing HF in the general population is approximately 1 in 5 for a person aged 40 years.[5] Adjusting for age, the incidence of HF has remained stable over the last 20 years, but the prevalence continue to increase.[5] One of the largest drivers on the increasing burden of HF in the developed world is ischaemic heart disease (IHD).[6] HF is associated with a high mortality with an estimated 30-40% mortality rate within the first year of diagnosis.[7] However, there is an improving trend in mortality demonstrated by the six month survival rate decreasing from 26% in 1995 to 14% in 2005.[8] The HF burden has implications for national health systems as it accounts for 5% of all acute medical and geriatric hospital admissions and is the commonest hospital admission cause in the over 65 year old population. It is estimated that hospital admissions due to HF will rise by 50% over the next 25 years.[4,9] The burden has eased slightly with an age–adjusted hospitalisation rate having decreased by 1–1.5% per annum since 1992/1993.[10] The improvements in mortality and hospitalisation is due to more effective treatments,[2,11-14] however the burden of the aging population and improved survival from HF means it remains a significant problem.

1.1.1 Reduced Ejection Fraction Heart Failure and Cardiac Dyssynchrony

Many patients with HF with reduced ejection fractions (HFrEF) develop dyssynchronous contraction of the heart due to damage of the underlying conduction tissue leading to inefficient cardiac contraction that leads to symptoms. Cardiac dyssynchrony is a complex and multifactorial process which impacts function.[15] Prolongation of the atrioventricular interval, can encroach on the starting of systole and filling within early diastole.[15] Ventricular contraction being delayed, the left ventricle (LV) diastolic pressures will exceed the left atrial pressure during passive filling, leading to the development of functional mitral regurgitation (MR).[15] The impact of reducing ventricular pre-load, leads to a reduction in LV contractility, by the Starling mechanism.[15] Moreover, the occurrence of intra- and inter-ventricular conduction delays leads to asynchronous contraction on the regional wall segments (ventricular/mechanical dyssynchrony), which leads to reduced stroke volume, LV ejection fraction (LVEF) and systolic blood pressure.[15] Ventricular dyssynchrony leads to incoordination of papillary wall contraction and further contributes to the development and progression of functional mitral regurgitation.[15] The development and progression of this process leads to or contributes to LV adverse remodelling.[15]

1.2 CARDIAC RESYNCHRONISATION THERAPY

Cardiac Resynchronisation Therapy (CRT) or 'biventricular pacing' involves implanting pacing leads into the heart to pace the left and right heart. The pacemaker leads are implanted via the transvenous route into the right ventricle (RV) and a branch of the coronary sinus (venous drainage of heart) to pace the LV , to resynchronise ventricular contraction. A lead

is implanted into the right atrium (RA) to achieve atrioventricular synchrony, this lead is not necessarily implanted in patients where pacing is not possible (e.g. Chronic Atrial Fibrillation). **Figure 1.1** demonstrates implantation of the CRT pacing leads. CRT can 'resynchronise' cardiac contraction through restorations of inter- and intra- ventricular and atrioventricular dyssynchrony.[15] Resynchronisation induces reverse LV remodelling by improving LVEF, contractility and LV filling time.[15]

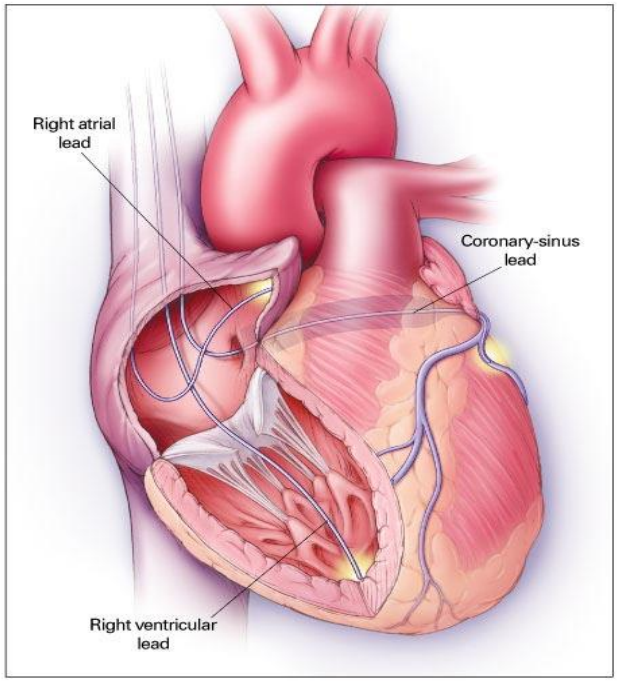


Figure 1.1 Cardiac Resynchronisation Therapy. Figure taken from Hare J *et al*.NEJM, 2002[16]

1.2.1 The Cost of CRT

CRT implantation is a costly intervention with a large up-front cost of an estimated £3,411 for a CRT Pacemaker (CRT-p) and £12,293 for a CRT Defibrillator (CRT-d).[17] Additionally there are ongoing costs of monitoring and replacement of pulse generators of CRTs.[18] The up-

front cost is larger than for many other medical devices.[18] Randomised Control Trials (RCT) have been used to model the quality adjusted life year (QALY) costs of a CRT. It is widely accepted that this falls below $50,000 per QALY which is the accepted cost of an intervention in the USA, and equates to approximately £39,000.[18] Efforts have focused on minimising this cost, by better identifying the CHF population who will benefit, streamlining implantation and distance monitoring to reduce patient visits to hospital [18]. However, the burden of cost to the healthcare system will continue to rise with the growing population of CHF patients who might benefit from CRT.

1.2.2 The Evidence for CRT

Over the last 25 years CRT has become one of the most effective treatments for CHF and is applicable to an estimated 25-30% of CHF patients.[19] In 1994 Cazeau *et al,*[20] demonstrated a four lead pacemaker in a 54 year old advancing HF patient improved New York Heart Association (NYHA) symptoms classification. Since then multiple RCTS have demonstrated the benefit of CRT for HFrEF patients with mechanical Dyssynchrony; reduced mortality and hospitalisation,[21-23] alongside improved quality of life (QoL),[23-27] symptoms,[21,28,29] functional performance[28,30] and LV volumes.[25,29,31] **Table 1.1** summarises the main RCTs demonstrating the benefit of CRT on HFrEF patients.

Table 1.1 Randomised control trials evaluating CRT in sinus rhythm (Adapted[15]).

Trial (ref)	Pts	Study Design	Inclusion	Outcome	Main Findings
MUSTIC-SR 2001 [21]	58	Single-blinded, crossover, randomised - CRT-p vs. OMT, 6months	NYHA III, LVEF<35%, QRS ≥150msec	**1°:** 6MWD **2°**: NYHA, QoL, peak VO2, LV volumes, hospitalisations, mortality	**CRT-p** ↑6MWD, ↓NYHA, ↑QoL, ↑peak VO_2 & **reduced** LV volumes, MR, hospitalisations
MIRACLE 2002 [28]	453	Double-blinded, randomised CRT vs. OMT, 6 months	NYHA III-IV, LVEF≤35%, QRS ≥130msec	**1°**: NYHA, 6MWD, QoL **2°**: Peak VO^2, LVEDD, LVEF, MR, Clinical Composite response	**CRT-p improved** 6MWD, NYHA, QoL, LVEF & **reduced** LVEDD, MR
MIRACLE-ICD 2003 [30]	369	Double-blinded, randomised CRT-d vs. ICD, 6 months	NYHA III-IV, LVEF≤35%, QRS ≥130msec	**1°:** NYHA, 6MWD, QoL **2°:** Peak VO2, LVEDD, LVEF, MR, Clinical Composite response	**CRT-d improved** NYHA, QoL, peak VO2
CONTAK-CD 2003 [25]	490	Double-blinded, randomised CRT-d vs. ICD, 6 months	NYHA II-IV, LVEF≤35%, QRS ≥120msec	**1°:** Clinical Composite **2°:** NYHA, 6MWD, QoL, peak VO2, LV volume, LVEF	**CRT-d improved** peak VO2, 6MWD & **reduced** LVEF
MIRACLE-ICD II 2004 [29]	186	Double-blinded, randomised CRT-d vs. ICD, 6 months	NYHA II, LVEF≤35%, QRS ≥130msec	**1°:** Peak VO2 **2°:** VE/VCO_2, NYHA, QoL, 6MWD, LVEF, LV Volumes, Clinical Composite	**CRT-d improved** NYHA, VE/VCO^2, LVEF & **reduced** LV volumes
COMPANION 2004 [22]	1520	Double-blinded, randomised - OMT vs. CRT-d /or CRT-p , 15 months	NYHA III-IV, LVEF≤35%, QRS ≥120msec	**1°:** All-cause mortality or hospitalisations **2°:** All-cause mortality, cardiac mortality	**CRT-d & CRT-p reduced** all-cause mortality & hospitalisation
CARE-HF 2005 [23]	813	Double-blinded randomised - OMT vs. CRT-p 29.4 months	NYHA III-IV, LVEF≤35%, QRS ≥120msec	**1°:** All-cause mortality or hospitalisations **2°**:All-cause mortality, NYHA, QoL	**CRT-p reduced** all-cause mortality, hospitalisations & **improved** NYHA, QoL
REVERSE 2008 [31]	610	Double-blinded, randomised - CRT-ON vs. CRT-OFF, 12 months	NYHA I-II, LVEF≤40%, QRS ≥120msec	**1°:** % worsened HF clinical composite **2°**: LVESVi, HF hospitalisations, All-cause mortality	**CRT-p/CRT-d did not change** the primary endpoint, **reduced** LVESVi, HF hospitalisations
MADIT-CRT 2009 [27]	1820	Single-blinded, randomised - CRT-d vs. ICD, 12 months	NYHA I-II, LVEF<30%, QRS >130msec	**1°:** All-cause mortality or HF hospitalisations **2°:** All-cause mortality, LVESV	**CRT-d reduced** the primary endpoint & LVESV, **CRT-d did not reduce** All-cause mortality
RAFT 2010 [26]	1798	Double-blinded, randomised - CRT-d vs. ICD, 40 months	NYHA II-III, LVEF<30%, QRS >120msec	**1°:** All-cause mortality or HF hospitalisations **2°:** All-cause mortality & CV death	**CRT-d reduced** primary endpoint **CRT-d (NYHA III) reduced** All-cause mortality

6MWD = Six Minute Walk Distance, CARE-HF = Cardiac Resynchronization Heart Failure, CONTAK-CD = CONTAK-Cardiac Defibrillator, COMPANION = Comparison of Medical Therapy, Pacing and Defibrillation in Heart Failure, MADIT-CRT = Multicenter Automatic Defibrillator Implantation Trial with Cardiac Resynchronization Therapy, MIRACLE = Multicentre InSync Randomized Clinical Evaluation, MIRACLE-ICD= Multicentre InSync ImplanTable Cardioverter Defibrillator trial, MUSTIC =Multisite Stimulation in Cardiomyopathies, RAFT=Resynchronization-Defibrillation for Ambulatory Heart Failure, 1° = Primary Outcome, 2°= Secondary Outcome

The RCTs for CRT represent benefit from CRT for patients with significantly reduced LVEF (≤35%), symptoms despite optimal medical therapy and a prolonged QRS duration (≥120msec) on resting electrocardiogram in sinus rhythm (**Table 1.1**). The detailed analysis of all the RCTs, related systematic review, sub-group analyses and the observational studies refines the groups of HFrEF patients that derive the most benefit and influence response to CRT. This evidence is the basis of the international CRT implantation guidelines and the weighting given to the evidence.

1.2.3 Left Ventricular Ejection Fraction and CRT

Studies have only examined patients with severe LV systolic dysfunction (LVEF <30-40%), with the specific exception for patients who have a bradycardia pacing indication and whether a CRT should be implanted over an simple RV pacing only device. Consistent trends suggest that sustained RV pacing induces deterioration in LV systolic function, therefore anticipating the deterioration with a CRT might prevent this.[15,32-34] The biventricular versus right ventricular pacing in patients with AV block (BLOCK HF) trial[32] was the largest (n=691) RCT examining RV pacing versus CRT on composite outcomes (all-cause mortality, HF hospitalisation ↓≤15% LV end systolic volume indexed to body surface area (m^2)(LVESVi)) for patients with LVEF ≤50% (mean = 40%) with atrioventricular node block. BLOCK-HF demonstrated following a 37 month observation period that patients undergoing a CRT implantation had a 26% greater reduction in outcome occurrence, although it should be noted some of the echocardiogram data was missing, so censoring of these patients occurred, excluding end-points from this study.[32] The evidence demonstrated that in patients with moderate /severe LV systolic dysfunction and a pacing indication a CRT could

be implanted in stead of a pacemaker when weighed against the small risks of the procedure. [32] Comparisons between RV pacing and CRT implantation for preserved systolic function demonstrated no statistical difference.[35,36]

1.2.4 New York Heart Association Symptom Classification and CRT

The strength of evidence around CRT implantation for all NYHA symptom classification is highly variable. The trials tended to recruit a higher proportion of NYHA II/III patients dependent on the specific inclusion criteria (**Table 1.1**). In the mild / no symptom trials (NYHA I-II) with severe LV systolic dysfunction (LVEF<30-40%), QRS duration ≥120-130msec improvement in cardiovascular outcomes and reverse LV remodelling was demonstrated.[27,29,31] NYHA I patients represented a small proportion (<20%) of the participants in all trials and showed a trend towards a benefit to improving cardiovascular outcomes.[27,29,31] Moreover, NYHA IV patients were under represented in the RCTs, representing between 7% and 15%.[15] The evidence for specific outcomes for NYHA IV patients with LV systolic dysfunction (LVEF<30-40%) and QRS duration ≥120-130msec is limited. One retrospective cohort study observed that 5 years survival was 40.4% following CRT, however this was based upon only 5 patients (n=723).[37]

1.2.5 QRS Duration and CRT

QRS duration is the most powerful predictor of benefit and response when a patient has a CRT implanted. Sub-group analyses of the MADIT-CRT[27,38] REVERSE[31] and RAFT[26] RCTs consistently demonstrate that patients with the greatest reduction in cardiovascular

outcomes are those with a QRS duration ≥150msec. These trials represent patients in NYHA class I-III and LVEF≤30-40%. Cleland *et al*[39] performed a large meta-analysis of individual patients (n=3782) from 5 Medtronic Ltd (Minneapolis, USA) sponsored RCTs (MIRACLE,[28] MIRACLE-ICD,[30] CARE-HF,[23] REVERSE[31] and RAFT[26]) comparing either CRT with OMT[23,28,31] or CRT-d with implantable cardiac defibrillators (ICD).[26] Pre-defined variables were examined for their ability to predict CRT cardiovascular outcomes (composite all-cause mortality/HF admissions or all-cause mortality alone).[39] For uniformity of the review, patients with atrial fibrillation (AF) and NYHA I symptoms had individual records removed from the analysis as they were only present in a small proportion of patients within one RCT cohort. Cleland *et al*[39] accounted for the influence of having an actual CRT implanted and treated it as a fixed affect variable in the prediction models. Increasing QRS duration was shown to be beneficial for patients undergoing CRT and predicted a reduction in cardiovascular outcomes.[39] **Figure 1.2** shows the hazard ratio (HR) and 95% confidence interval (CI) for CRT vs. control for the composite of all-cause mortality or HF hospitalisation plotted against QRS duration. Cleland *et al*[39] demonstrate a greater magnitude of benefit from incremental increases in QRS duration following CRT implantation improving outcomes. The definitive benefit was observed at ≥140msec (CI lines cross the HR 1.0).[39] The benefit reached a plateau beyond 180msec for composite outcome alone.[39] Interestingly left bundle branch block (LBBB) morphology was associated with broader QRS durations, generating the question of whether the observed predictive power of QRS duration was due to this cofounding factor. Cleland *et al*[39] demonstrated non-LBBB had an increased trend towards higher mortality, however when QRS duration was removed from the multivariable prediction model, little difference was noted between LBBB and non-LBBB in terms of impact on mortality. [39] The

Cleland *et al*[39] meta-analysis is powerful due to the access to individual participant records across 5 RCTs. Other reviews quoted in this body of work are aggregate reviews and do not account for individual study confounders. However, only Medtronic Ltd sponsored RCTs were included, which the authors do acknowledge as a major limitation.

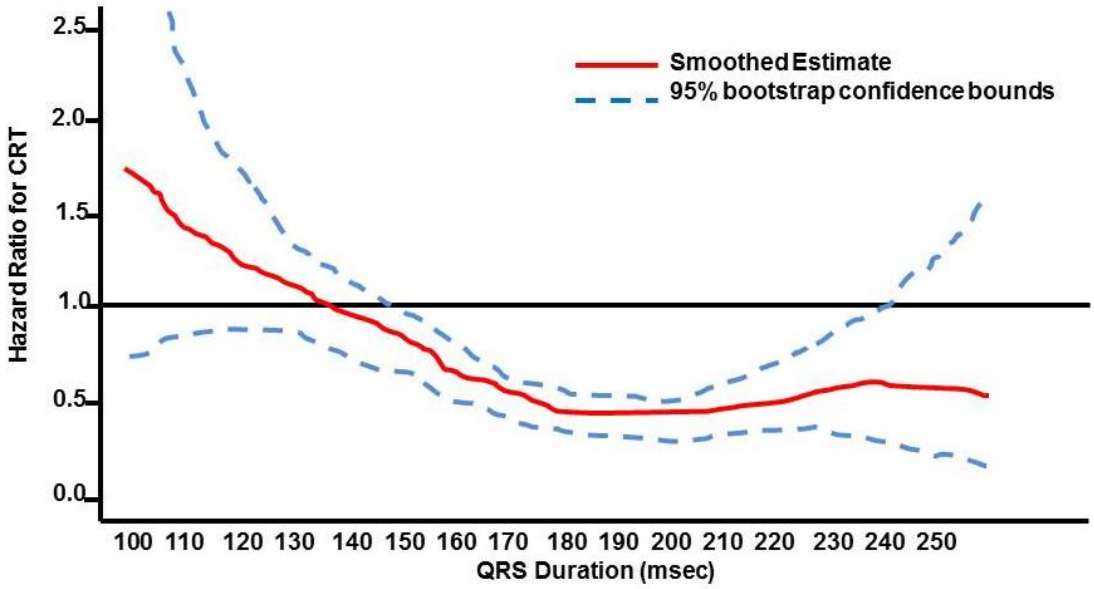


Figure 1.2 Relationship of CRT on all-cause mortality or HF hospitalisation and QRS duration. Models demonstrating Hazard Ratio's and 95% confidence interval for CRT vs. controls (OMT/ICD/Back up pacing) against the QRS duration. (Adapted[39])

There is definitive evidence demonstrating that patients with a QRS duration 120-129msec do not benefit from CRT implantation. In 2007, the 'CRT IN Patients with Heart Failure and Narrow QRS' (RethinQ) trial[40] recruited patients (n=172) with standard implantation criteria with QRS complexes $\leq$130msec and cardiac dyssynchrony, randomising participants to therapy CRT-ON or CRT-OFF (after implantation). Over six months, the CRT-ON group demonstrated an improvement in peak O_2 consumption (peak VO_2) (p=0.02), however in a

pre-defined subgroup analysis those with a QRS<120msec demonstrated no difference (p=0.45).[40] The RethinQ[40] RCT observation period was too short to be able to examine the impact of biventricular pacing on a narrow QRS upon morbidity and mortality. More recently, 'the Cardiac Resynchronization Therapy in Heart Failure with a Narrow QRS' Complex (Echo-CRT) trial[41] enrolled patients (n=855) across 115 centres who met standard implantation criteria and had a QRS complex ≤130msec with evidence of cardiac dyssynchrony. Following CRT implantation they were randomised to either CRT-ON or CRT-OFF to examine impact on all-cause mortality and HF hospitalisation (treated as a composite outcome). The Echo-CRT[41] RCT was stopped early on '*the basis of futility with a potential for harm*'. The trial demonstrated CRT-ON had a higher rate of composite primary end-points occurring compared to CRT-OFF group (28.7% vs. 25.2%), which was not statistically significant (p=0.15).[41] However, when all-cause mortality was examined on its own CRT-ON, had a statistically higher occurrence than CRT-OFF (11.1% vs. 6.4%, p=0.02).[41] The definitive benefit of CRT is in those with a widened QRS, despite the presence of mechanical cardiac dyssynchrony.

1.2.6 QRS Morphology and CRT

QRS morphology has been demonstrated to be important in determining response to CRT implantation. Subsequent sub-group analyses of the MADIT-CRT[42], RAFT[26] and REVERSE[43] trials all identified complete LBBB demonstrated a better outcome on the composite of all-cause mortality and hospitalisation compared with right bundle branch block (RBBB) and non-specific intraventricular conduction delay (NIVCD). In 2012 Sipahi *et al*[44] performed a large meta-analysis (n=5,356) examining CRT RCTs that reported clinical outcomes (all-cause

mortality/HF hospitalisations) corresponding with Bundle Branch Block (BBB) morphology. COMPANION[22], CARE-HF[23], MADIT-CRT[42] and RAFT[26] were the only trials that met the eligibility criteria.[44] Sipahi *et al*[44] demonstrated a highly significant reduction in the composite outcome for patients undergoing CRT implantation with complete LBBB (Risk Ratio (RR) 0.64, CI (95%) 0.52-0.77, $p<0.0001$). Within the included RCTs of the meta-analysis, patients with LBBB tended to have wider QRS's, which may have confound the results.[44]

Cunnington *et al*[45] in a meta-analysis of six landmark CRT RCTs[22,23,26-28,31] analysed 6914 participants and compared those with and without LBBB (Non-LBBB was a classification for QRS morphology in four included trials[22,26,27,31] and RBBB was used in the other two RCTs [23,28]). The two trials which classified non-LBBB as RBBB were not involved in the sensitivity analysis.[45] NIVCD was accounted for, in the definition of BBB in four trials.[23,26,27,31] The review summarises that the six trials represented participants with NYHA I-IV, LVEF$\leq$30-40% and QRS$\geq$120msec.[45] **Table 1.1** summarises the characteristics of all the included studies. Cunnington *et al*[45] demonstrated no benefit from CRT for patients with non-LBBB QRS morphology for a pooled outcome of all-cause mortality and HF hospitalisation (HR 1.09, CI (95%) 0.85 – 1.39). It should be noted that Cunnington *et al*[45] only studied cardiovascular end-points and did not examine symptom, functional or echocardiographic outcomes. It is also acknowledged that NYHA classes I and IV are underrepresented in the RCTs and the observations are driven by those with class II and III symptoms. The MADIT-CRT trial[27] enrolled 536 non-LBBB participants with NYHA I-II. This RCT demonstrated no clinical benefit from CRT for non-LBBB patients and actually showed it increased the risk of mortality (HR

1.57, CI (95%) 1.03-2.39).[46] A meta-analysis by Sipahi *et al*[44] also demonstrated there was no benefit to implanting CRTs in patients with non-LBBB. The challenge remains how to treat those with non-LBBB with a widen QRS, which remains an active indication for implanting a CRT.[15,17,47] Cleland *et al*[39] in a large meta-analysis observed that non-LBBB did not predict cardiovascular outcomes when QRS duration was removed from the analysis. Different BBB patterns have been demonstrated on recent electrocardiographic activation mapping studies to have heterogeneous patterns and should be considered as different entities,[24,48] whereas QRS durations can be considered on a continuous spectrum with incremental benefit the wider the duration when a CRT is implanted.[39] Currently QRS duration represents the most powerful predictor and BBB morphology should be considered separately with LBBB being more favourable for a successful outcome.

1.2.7 Atrial Fibrillation and CRT

AF commonly co-exists in patients with HF and its presence can reduce the success of CRT.[49] Understanding the true influence of AF on the success of CRT is difficult as patients with AF tend to be older, have more co-morbidities and are more unwell. Comparison between sinus rhythm and AF is influenced by these confounding factors, which often infer worse prognosis.[15] AF is underrepresented in CRT RCTs and reliance is needed upon meta-analyses. It has been observed that AF patients receiving CRT have a similar improvement in LVEF compared with those in sinus rhythm, but have worse symptom and functional response.[49] In a large (n=7495) meta-analysis of 33 observational studies, Wilton *et al* [49] compared those with AF (22.5%) to sinus rhythm receiving CRT and observed a significantly higher all-cause mortality and non-responder rate in the AF group. Evidence on the precise

recommendations for CRT in patients with AF remains weak and is based on limited evidence and expert opinion. However, implantation is favoured if >99% biventricular pacing percentage can be achieved.[15]

1.2.8 Cardiac Dyssynchrony and CRT

One of the recent significant changes to the international guidelines was the removal for the need to demonstrate cardiac dyssynchrony on echocardiography if the patient's QRS duration is 120-149msec on resting ECG. The CARE-HF trial[23] eligibility criteria required patients with a QRS duration of 120-149 msec to have cardiac dyssynchrony demonstrated on echocardiography. Cardiac dyssynchrony was defined as achieving two of three criteria: aortic pre-ejection delay >140msec, interventricular mechanical delay (IVMD) of 40msec, or delayed activation of the posterolateral LV wall. The IVMD was calculated as the time difference between the onset of forward flow in the Aortic pre-ejection time (APET) and Pulmonary pre-ejection time (PPET) outflow tracts (IVMD = APET – PPET).[23] A sub-group analysis of CARE-HF demonstrated those patients with an IVMD ≥49.2msec implanted with a CRT had a reduced composite outcome (all-cause mortality or hospitalisation for a major cardiovascular event) (HR 0.50, CI (95%) 0.36-0.70).[23] A pre-defined sub-study[50] of CARE-HF[23] observed that patients with IVMD (≥49.2msec) benefitted more from CRT from a greater reduction in the composite outcome (HR 0.99, 95% CI 0.98–1.00). The demonstration of the benefit of CRT on patients who demonstrated cardiac dyssynchrony (as per CARE-HF) formed a part of the implantation guidelines.[51]

Cardiac dyssynchrony as an indicator of CRT success was seen sceptically, as the data was based upon a sub-group analysis of CARE-HF.[23] These suspicions were validated by the

results of the prospective, multicentre Predictors of Response to Cardiac Resynchronization Therapy (PROSPECT) study.[52] PROSPECT recruited 426 participants who successfully had CRTs implanted across 53 international centres with standard CRT implantation indications to measure the ability of 12 pre-defined echocardiographic measures of cardiac dyssynchrony (including IVMD) abilities to predict response, alongside their validity and reliability as measurments.[52] Two definitions of response were utilised at six months follow-up; a clinical (HF clinical composite score) and echocardiographic (>↓15% LVESV).[52] The 12 predefined cardiac dyssynchrony measures demonstrated wide variability in their ability to predict clinical and echocardiographic response. For echocardiographic response prediction, the sensitivity ranged from 9% to 77% and specificity from 31% to 93%.[52] None of the cardiac dyssynchrony variables achieved an area under the receiver operating curve (ROC) for either response definition ≤0.62; representing a poor ability to discriminate response.[52] PROSPECT also identified a high variability between operators to accurately reproduce cardiac dyssynchrony measurements.[52] Notably only 286 participants had paired baseline and six month follow-up echocardiogram completed successfully, due to a combination of poor quality images, presence of AF and mortality events.[52] The results of PROSEPCT demonstrated weak predictive power and high inter-operator variability of cardiac dyssynchrony echocardiographic markers when used in multiple centres.[52] Currently other echocardiographic measurements of cardiac dyssynchrony continue to be researched, echocardiographic strain analysis offers some future potential,[53-55] however for now the best discriminator is QRS duration on resting ECGs and this is now used in the current guidelines.[15,17,47]

1.2.9 Cardiac Resynchronisation Therapy Implantation Criteria

The current evidence has been significantly modified over the last 15 years of implantation as more evidence has been produced. The previous section demonstrated the evolution and refinement of the current evidence, which reflects the current International guidelines (European Society of Cardiology (ESC)[15] and American Heart Association(AHA)[47]). These guidelines have recently changed to reflect more recent evidence including now implanting patients with AF and bradycardia pacemaker indications.[15,47,56,57] In June 2014, in the UK, NICE revised guidance on CRT implantation that reflect the updated international guidelines.[17] Current indications are for CHF (LVEF <35%) with NYHA II/III/IV symptoms on optimal medical therapy (Angiotensin Converting Enzyme, Beta-Blocker +/- Mineralocorticoid Antagonist) with a QRS duration on resting ECG with either: 120-149msec with LBBB or ≥150msec duration. Patients in atrial fibrillation who can be rate controlled (medication or AV node ablation) and fulfil the CRT criteria should be considered for a CRT.[47] Patients (LVEF <35%) who are anticipated to require ventricular pacing >40% of the time should be considered for CRT.[47] **Table 1.2** summarises the NICE 2014 CRT implantation guidelines.

Table 1.2 NICE 2014 (TA314) Indications for Implantable Cardiac Defibrillator and CRT in Patients with LVEF≤35%. (Adapted[17])

	NYHA Class			
QRS Interval	**I**	**II**	**III**	**IV**
<120 msec	ICD if there is a high risk of sudden cardiac death			ICD and CRT not clinically indicated
120-149msec without LBBB	ICD	ICD	ICD	CRT-p
120-149msec with LBBB	ICD	CRT-d	CRT-p or CRT-d	CRT-P
>150msec	CRT-d	CRT-d	CRT-p or CRT-d	CRT-P

The ESC introduced new recommendations for CRT implantation in August 2016 at their annual conference.[58] Referring to the issues raised by the strength of evidence about implanting CRT into patients with a low QRS 120-130msec raised by Cleland *et al*[39] and the ECHO-CRT[41] RCT the new guidelines recommend CRT should only now be implanted with a QRS≥130msec. The NICE 2014[17] guidelines (**Table 1.2**) remain applicable currently, however this is likely to change in the near future.[58]

1.2.10 UK Cardiac Resynchronisation Therapy Implantation

Over the last decade several hundred thousand CRTs have been implanted worldwide.[19] In 2013 the UK was the fourth highest total CRT implanter in Western Europe.[59] **Figure 1.3** demonstrates the increasing CRT implantation year-on-year in the UK, over the last 10 years. Scotland's national implantation figures are not presented as there was an incomplete dataset provided to the national audit of cardiac rhythm management.[59] These figures represent a broadening of the international/national implantation guidelines as the evidence has become more extensive and refined. **Figure 1.3** compares the changes in the national and international implantation criteria with UK procedure rates. The increasing

implantation rates also represent increasing number of secondary care centres in the UK to implanting CRTs.[51]

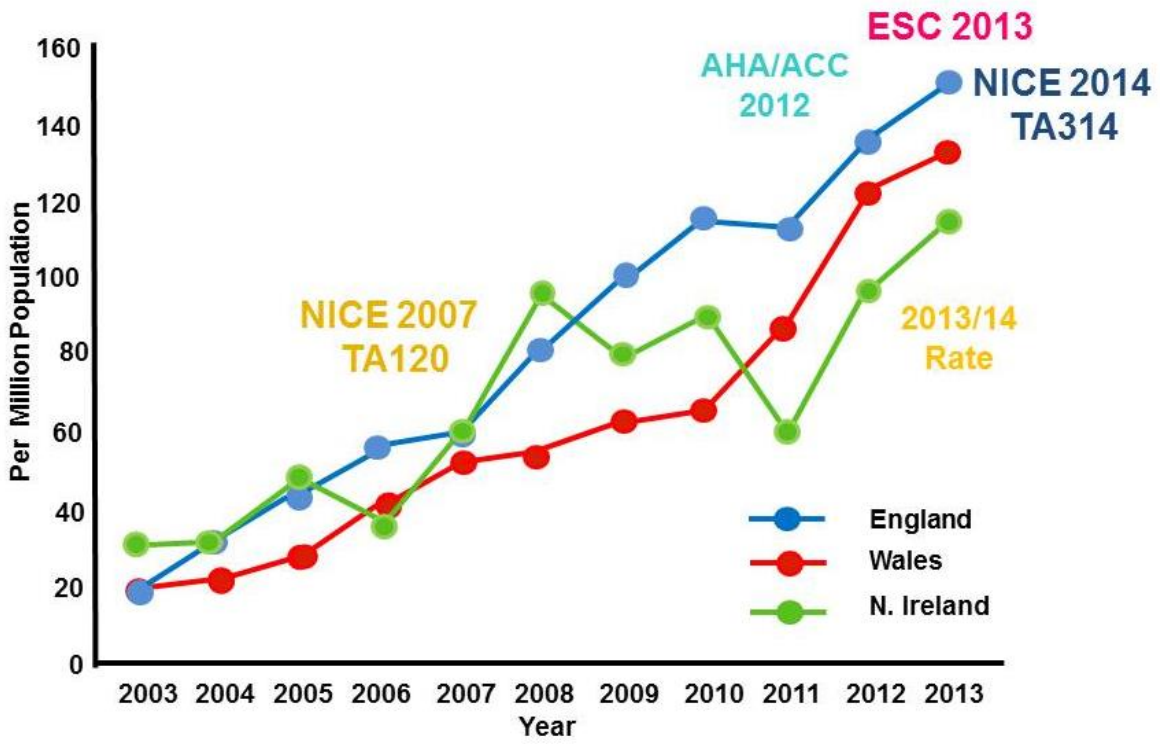


Figure 1.3 United Kingdom total CRT implant rates 2003-2014 compared with release in national and international implantation criteria. The 2013/2014 years are combined as the data collection changed to reflect a March to March data collection period. (Adapted[59])

1.3 CRT NON-RESPONSE

Despite two decades of refined RCTs and detailed observational trials examining those patients that benefit from CRT, there remains an unchanging minority of 20-40% of HFrEF patients that meet criteria for CRT placement but fail to respond.[22,23,26,27,60] QRS duration and morphology have consistently been demonstrated to be the strongest predictors of cardiovascular outcomes, as summarised in the earlier discussions.[39] Cleland *et al*[39]

demonstrated the incremental strength of increasing QRS above 140msec to predict improved outcomes (**figure 1.2**). QRS morphology does not demonstrate such clear strength to predict response,[39] though it is clear the non-LBBB morphology favours poorer response.[45] Questions still remain around the benefit of CRT in patients with QRS durations 120-140msec and the additional benefit LBBB morphology offers.[45] Calls have recently been made for an RCT of patients with CRT already in-situ to have their devices de-activated for a period of observation who have a narrow QRS and/or non-LBBB to examine for any benefits.[61,62] Moreover the apparent influence of BBB morphology on QRS duration >150msec remains unclear.

Beyond QRS durations and BBB morphology, many other predictors of response to CRT have been identified. Sub-studies of the large CRT RCTs have identified multiple variables that improve morbidity and mortality. In a sub-study of the MADIT-CRT trial,[27] Hsu *et al*[38] performed a best-subset regression on patients whom had paired echocardiograms at 12 months and had been assigned to have a CRT-d (n=752) to examine for predictors of echocardiogram super-responders (top quartile of LVEF change). Six predictors were identified as being associated with LVEF super-response; female gender (Odds Ratio (OR) 1.96, p=0.001), no prior myocardial infarction (MI) (OR 1.80; p<0.01), QRS duration ≥150msec (OR 1.79, p<0.01), LBBB (OR 2.05, p<0.01), body mass index (BMI) <30 kg/m2 (OR 1.51, p=0.035), and smaller baseline left atrial volume index (OR 1.47, p=0.001).[38] The impact of the CRT was not accounted for in this analysis by Hsu *et al,*[38] further this was a sub-study analysis and to be included there had to be paired echocardiograms demonstrating a selection bias towards participants that had survived to that time.

Cleland *et al*[39] examined multiple predefined potential predictor variables (age, gender, NYHA class, HF aetiology, QRS morphology, QRS duration, LVEF, and systolic blood pressure) and only QRS duration demonstrated the ability to predict CRT outcomes. Cleland *et al*[62,63] consistently make the argument that much of the evidence for predictors is based upon sub-group analyses. Moreover, in these subset studies and meta-analyses based on the CRT RCTs the impact of the device is not accounted for and confounds the results observed.[62,63]

Many observational studies have been performed in the intervening two decades to examine the potential of different variables to predict CRT response/outcome. These observational studies tend to be of limited value, often being under-powered, have flaws in their study methodology, not accounting for CRT implantation and using a variety of different response/outcome definitions.[63] Their value shadows that of the often quoted CRT RCT sub-studies and more importantly meta-analyses. However, observational studies tend to emphasise the value of response in terms of patient centred criteria (symptoms, function and quality-of-life) compared to the majority of CRT trials with their composite cardiovascular outcomes (**Table 1.1**). There are also well conducted observational studies that often generate new lines of hypothesis and investigation; therefore they still have value in the investigation of non-response. **Table 1.3** summarises important observational studies examining clinical predictors.

Table 1.3 Summary of observational studies examining pre-implant 'predictors' of CRT response

Trial (ref)	Patients	Study Design	Inclusion	Response Criteria	Main Findings
Diaz-Infante *et al* 2005 [64]	143	Prospective Observational Study; Multicentre (Spain); All successful CRT implants; 6 months	NYHA II-IV, LVEF ≤45%, QRS ≥130msec	**Clinical:** ↑≥10% 6MWD & Survived & No Heart Transplant	**Predictor Non-Response:** Ischaemic Aetiology, LVEDD (≥75mm) & severity of MR
Shanks *et al* 2011 [65]	581	Observational study ; Single centre (Holland); 6 months	Not clear which NYHA, LVEF≤35%, QRS≥120msec	**Clinical & Echocardiographic:** ↓NYHA ≥1 & survival & no heart transplantation ↑>15% LVESV	**Predict Non-Response:** Ischaemic Aetiology, Shorter 6MWD at baseline, less baseline cardiac dyssynchrony & LV lead position
Lin *et al* 2014 [66]	193	Retrospective Observational Study (China); Single centre; All consecutive CRTs; 12 months	NYHA II-IV, LVEF≤35%, QRS≥120msec	**Echocardiographic:** ↑≥5%LVEF & survived & being free from HF hospitalisation	**Predicts Non-response:** non-LBBB & non-optimal LV lead position
Rinkuniene *et al* 2014 [67]	82	Retrospective Observational Study; Single centre (Lithuania); 12 months	NYHA III-IV, LVEF≤35%, QRS≥120msec, LBBB	**Clinical:**↓≥1 NYHA & ↑>15% 6MWD **Echocardiographic:** ↑≥15% LVESV	**Predicts Response:** Non-Ischaemic Aetiology (Clinical) & LVEDD (≤75mm) (Echo)
Sassone *et al* 2015 [68]	243	Retrospective Observational Study; All consecutive CRTs; Majority CRT-d; Single centre (Italy); 6 months; LBBB in predictor analysis	NYHA II-IV, LVEF≤35%, QRS≥120msec	**Echocardiographic:** ↑>15% LVESV **Clinical Composite:** HF hospitalisation, mortality & first sustained VT	**Predict Non-Response:** Ischaemic Aetiology & QRS duration (≥178msec) **Clinical Composite:** non-LBBB ↑ rate events

Diaz-Infante *et al*[64] offered a large multicentre prospective observational study, which was not powered. There were 197 patients who originally met the eligibility criteria, however 54 (27.4%) were excluded (6-minute walk test (6MWT) not performed, CRT unsuccessful etc).[64] Interpretation of the results must be performed in the context that this study started recruiting in 2001 and with all the advances in technology and skills since then, this will have affected the results.[64] Critically the variables being tested in the prediction model were not pre-defined. In terms of observation study, this is a strong one given its prospective and multicentre design, but suffers from selection bias.

Shanks *et al*[65] undertook a large observational study, with short follow-up. From the report itself, it is unclear if this is prospective or retrospective in design with a small number of NYHA IV patients.[65] The variables being tested in the predictor model were not pre-specified; moreover multiple statistical testing was not corrected for.[65] The results support others observed in other studies, including the MADIT-CRT substudy.[38]

Lin *et al*[66] performed a non-powered observational study. The predictors that were tested were listed in the methods. LV geometric measures including LVEF were used as predictors of response; a difference between response and non-response was identified, this however was to expected given the 'response' definition echocardiographic variables (t**able 1.3**)[66] LV lead position was observed to be a predictor of non-response, however there is no discussion about selection of LV lead position selection and pacing programing involved. Interestingly pre-implant QRS duration is a strong univariate predictor of response (OR 1.02,

CI (95%) 1.01-1.04), *p*=0.001), however in multivariable analysis the association is lost (OR 1.00, CI (95%) 0.98-1.03, *p*=0.81).[66] Lin *et al*[66] observed responders had significantly larger QRS durations and more LBBB than non-responders.[66] Non-LBBB morphology was found to be a significant predictor of non-response. In the literature QRS duration is a more powerful predictor than bundle branch morphology, however in this study confounding effect of both variables is not adequately dealt with to determine the true effect.

Rinkuniene *et al*[67] was a single centre study observational study with small participant numbers and was not powered, therefore the observations made are questionnable. Furthermore there were a couple of other flaws with the study; it is somewhat surprising that all procedures were successful and variables examined as predictors were not predefined.[67] These factors suggest a flawed observation study that likely suffers from selection and reporter bias.

Sassone *et al*[68] present a large retrospective, single centre cohort study, which was not powered. They utilised two different outcome measures.[68] The focus of the study was on LBBB (59.7%) vs non-LBBB (n=74).[68] No pattern was noted with non-LBBB, however a 'U' shaped distribution was noted with LBBB with clusters on echocardiographic non-response clustered at $\leq$130msec and $\geq$180msec.[68] Despite this observation, BBB status was not tested as a predictor, but presumed to be.[68] The regression modelling was only performed on participants with defined LBBB. QRS durations $\geq$178msec was defined as being the best cut off to define echocardiographic non-response in patients with a CRT and LBBB. Sassone *et*

al[68] implies BBB is the greatest predictor, without testing it and goes on to look at additional factors like QRS durations.[68] Conversely, higher level evidence has demonstrated similar observations with the plateau effect of benefit to patients with a QRS >180msec, but this was separate to BBB morphology (**figure 1.2**).[39] Sassone *et al*[68] identified findings that had already been demonstrated by Cleland *et al*[39].

The observational studies discussed and summarised (**Table 1.3**) are not exhaustive, but reflect the most important published in conjunction with the sub-group studies of the CRT RCTs and the meta-analyses to investigate pre-implant factors that might predict response. The observational studies have several flaws that limit their value in drawing conclusions which can be implemented clinically. These observational studies do however offer insights into specific patient population's, reflect real world cohorts, and test response criterion beyond absolute endpoints. A criticism of the major CRT RCTs and meta-analyses is that they deal with response in terms of absolute outcomes and don't necessarily reflect those factors important to patients; e.g symptoms, exercise tolerance and QoL. Many of these observational studies do reproduce findings of other analyses. However, these findings are not reliably reproduced between different studies, resulting in a variety of variables being identified as potential predictos. QRS duration and BBB morphology are the most important and consistently reported factors that predict response/outcomes.[39] Additional factors like aetiology appear to be important as well in predicting response. Yet in spite of extensive investigation non-response has remained entrenched throughout the last two decades at 20-40%.[63]

1.4 DEFINING CRT RESPONSE

The consistent issue across all research examining CRT response is the variety of different definitions applied in the literature. This makes comparing and pooling data for comparison difficult due to the heterogeneity of the criterion used for response.[1,19] Fonwalt *et al*[1] performed a seminal systematic review of the 26 most cited papers on predicting CRT response and extrapolated 17 different criterion (**Table 1.4**). Fifteen criteria were all applied to the PROSPECT trial cohort (2 criteria could not be calculated).[1,52] The application of the different definitions demonstrated response varied between 32% and 91%.[1,52] All 15 criteria were compared to each other to measure the amount of agreement between definitions (105 combinations).[1] Overall 79 (75.2%) pairs of definitions had '*poor agreement*'.[1] Moreover, a strong association of agreement was only observed in 4 (3.8%) pairs of definitions. All echocardiographic and clinical definition combinations had a poor association.[1] Bleeker *et al*[69]observed 76% agreement when applying a clinical (↓≥1 NYHA classification) and (↓≥15% LVESV) to 144 consecutive patients undergoing CRT.[69] These results suggested a better clinical and echocardiography definition agreement than Fornwalt *et al*[1] had identified. Agreement was not tested in this analysis and the Bleeker *et al*[69] study was included in the Fornwalt *et al*[1] systematic review.

Table 1.4 Different CRT response definitions identified by Fornwalt *et al* (Adapted[1])

Echocardiographic Response criteria
1. ↑≥5% LVEF (absolute)[70,71] 2. ↑≥15% LVEF[72,73] 3. ↓ ≥10% LVESV and survived progressive HF by 6 months[74,75] 4. ↓>15% LVESV[52,71,76-80] 5. LVESV <115% of baseline[81] 6. ↓>15% LVESVI[82] 7. ↓>15% LVEDV[71] 8. ↑≥15% Stroke volume[73,83,84]
Clinical Response Criteria
9. ↓≥1NYHA[85-87] 10. ↓≥1 NYHA & survived progressive CHF by 6 months[88] 11. ↓≥1 NYHA & ↑≥25% 6MWD[89] 12. ↓≥1 NYHA & ↑6MWD ≥25% & survived progressive HF by 6 months[69,90] 13. ↑>10% 6MWT & no heart transplant & survived by 6 months [64] 14. (↓≥1 NYHA or ↑>10% peak $\dot{V}O_2$max or ↑>10% 6MWD) & survived & no HF hospitalisation[91] 15. 2 out of 3:[76] ↓≥1 NYHA ↑≥50metres 6MWT ↓≥15QOL 16. Clinical composite score improved[52]
Combined Response Criteria
17. (↑≥5% LVEF [absolute] or ↑≥30 m 6MWT) &(↓≥1 NYHA or ↓≥10 QOL)[92]

Agreement between the definitions is poor, even between similar categories of criteria.[1] A definition for response is still required to define success of CRT implantation. The problem

continues to persist in current practice. **Table 1.3** demonstrates this inconsistency in response definition application in observational studies over the last three years.[66-68] However, the combination of echocardiographic and clinical criterion to form a composite definition seem to have abated given the poor agreement between them.[1,66-68] In defining a response criteria, as Fornwalt *et al*[1] comments HF should be considered as a *'debilitating life-threatening disease'* where an effective treatment must improve '*symptoms, quality and duration of life'.* An effective definition should reflect these elements. Packer *et al*[93] in the seminal paper on HF composite scores, identified the pitfalls of using one individual metric to measure response, composite scores can minimise this problem. No universal response definition has yet to be agreed upon, however the consensus is a composite criterion is required. [93]

1.5 BIOMARKERS AND CARDIAC RESYNCHRONISATION THERAPY

CHF is a heterogeneous condition, which develops as a result of cardiac injury from a variety of potential mechanisms. The development and progression of HFrEF leads to profound changes at the macroscopic and molecular level in the body. Adverse cardiac remodelling leads to neurohormonal activation, pro-inflammatory changes, extracellular matrix remodelling, myocardial stress leading to apoptosis and cardiac fibrosis.[94] Unabated these processes contribute to the progression of adverse cardiac remodelling and poor outcomes from CHF. The identification of the alteration and progression of these processes has been observed through measuring related circulating biomarkers. Kimmenade[94] summaries many biomarkers related to these processes that are associated with HF development, prognosis and outcomes. CRT is applicable to 25-30% of patients with CHF and ever more

implantations are occurring both globally and within the UK. Yet non-response remains an unchanging and expensive problem for patients and the National Health Service.[15,18,59] Over the last 15 years circulating biomarkers have been studied to see if they may be potential predictors of CRT response. **Figure 1.4** demonstrates the increasing studies in the literature looking as CRT and biomarkers.

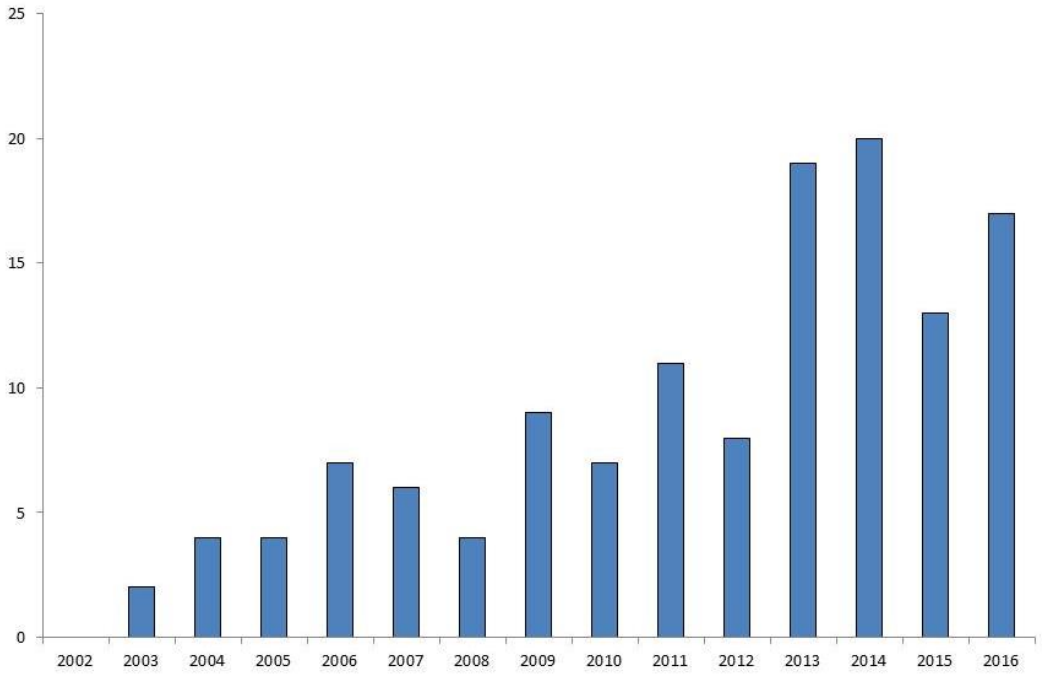


Figure 1.4 Number of publications on 'CRT and biomarkers' over 14 years. Search results from PubMed for 'Biomarker AND Cardiac Resynchronization Therapy'

1.5.1 Brain natriuretic Peptide

Brain Natriuretic Peptide (BNP) and its derivative (NT-*pro*-BNP) are released from cardiac myocytes in response to myocardial stress in relation to volume and pressure overload.[95] BNP was the first target to study as a potential circulating biomarker given its role in HF diagnosis and prognostication.[2] BNP has been demonstrated to alter following CRT. When

BNP levels decline it has been shown to be associated with reverse cardiac remodelling and improved outcomes.[96-99] BNP decline has been considered to be a superior response definition that others previously used.[100] Hoogslag *et al*[100] observed that change in BNP was a reasonable definition of response and compared to other definitions used.

The ability of baseline BNP to predict response has also been studied. Lellouche *et al*[101] performed a retrospective study on patients undergoing CRT and determined that a higher BNP at baseline was the strongest independent predictor of response. They determined that a BNP value >447 pg/ml demonstrated a sensitivity of 62% and specificity of 79% in identifying CRT response.[101] However, Berger *et al*[102] in a subgroup study of the CARE-HF trial identified that a lower median BNP at baseline was a predictor of better cardiovascular outcomes (sudden death and death from HF).[102]

Natriuretic peptides have been shown to be highly related to the clinical outcome and CRT response.[103] The challenge with the use of BNP and NT-*pro*-BNP is the high variability demonstrated in healthy patients and those with CHF.[103] In healthy people, the sequential change in BNP and NT-*pro*-BNP has been observed to up to 92% and 168% respectively.[104] This observation was also seen in stable CHF patients with a week to week intra-individual coefficient up to 35%.[105] This inherent individual variability is a challenge to BNP being a useful circulating biomarker of response.[103]

1.5.2 Growth Differentiation Factor-15

Growth Differentiation Factor-15 (GDF-15) is a member of the growth factor-ß cytokine superfamily and is implicated in the regulation of cell survival, proliferation and differentiation.[106] GDF-15 is also implicated in protection from ischaemia/ reperfusion injury.[107,108] In acute coronary syndrome patients GDF-15 demonstrated an ability to predict future risk of developing HF.[109] In the Valsartan Heart Failure (Val-HeFT) Trial[110] a raised GDF-15 was shown to be an independent predictor of poor HF outcomes, moreover it added prognostic value to other variables; NYHA, LVEF and NT-*pro*-BNP levels. The intra-individual variability of GDF-15 was been shown to be better than BNP/NT-*pro*-BNP.[111]

Foley *et al*[112] demonstrated in a single centre, prospective observation study, that GDF-15 is an independent predictor of mortality and morbidity in CHF patients (LVEF<35%) undergoing CRT.[112] When added to NT-*pro*-BNP the predictive value was additive.[112] However Foley *et al*[112] demonstrated that GDF-15 did not predict CRT response (survival & no HF hospitalisations; ↓≥1 NYHA class or ↑≥25% 6MWD at 1 year). GDF-15 was only powered to predict cardiovascular outcomes and not response.[112] Furthermore Foley *et al*[112] accounts for this result because of *'very high biological variability of these analytes'*. GDF-15 is reported to be better than BNP at predicting response, however questions still remain over its predictive valve. No more recent studies or trials have yet been performed on GDF-15 and should be considered in studies in the future.

1.6 EXTRACELLULAR CARDIAC MATRIX

The cardiac extracellular matrix (ECM) is a dynamic support structure that remodels following cardiac injury.[94,113] Progressive ECM remodelling is closely linked to the development of cardiac fibrosis.[113] The cardiac ECM remodelling is closely associated with the development, severity and progression of HF.[113] Biomarkers that highlight remodelling have been associated with HF outcomes.[94,113]

1.6.1 Cardiac Extracellular Matrix Structure

The heart consists of 30% myocardial cells and 70% non-myocardial support cells.[114] These support cells include; endothelial cells, fibroblasts, vascular smooth muscle cells and macrophages. The most common non-myocardial cells are fibroblasts', contributing 67% of the total. [114] These cells are critical to myocardial homeostasis. [114] Fibroblasts primary function is to maintain ECM homeostasis, including releasing signalling molecules, cytokines and growth factors.[114,115] Fibroblasts are responsible for production of the collagens.[116] Diverse cell types adhere to the ECM including fibroblasts, myocytes and endothelial cells.[114,115] The main structural protein is type I collagen but also includes type III, IV, V, VI, glycoproteins and proteoglycans.[115] Type I has high tensile strength and type III provides greater distensibility.[116] **Figure 1.5** demonstrates the cardiac ECM in the myocyte bundle and the interaction with the cardiomyocyte cell membrane.

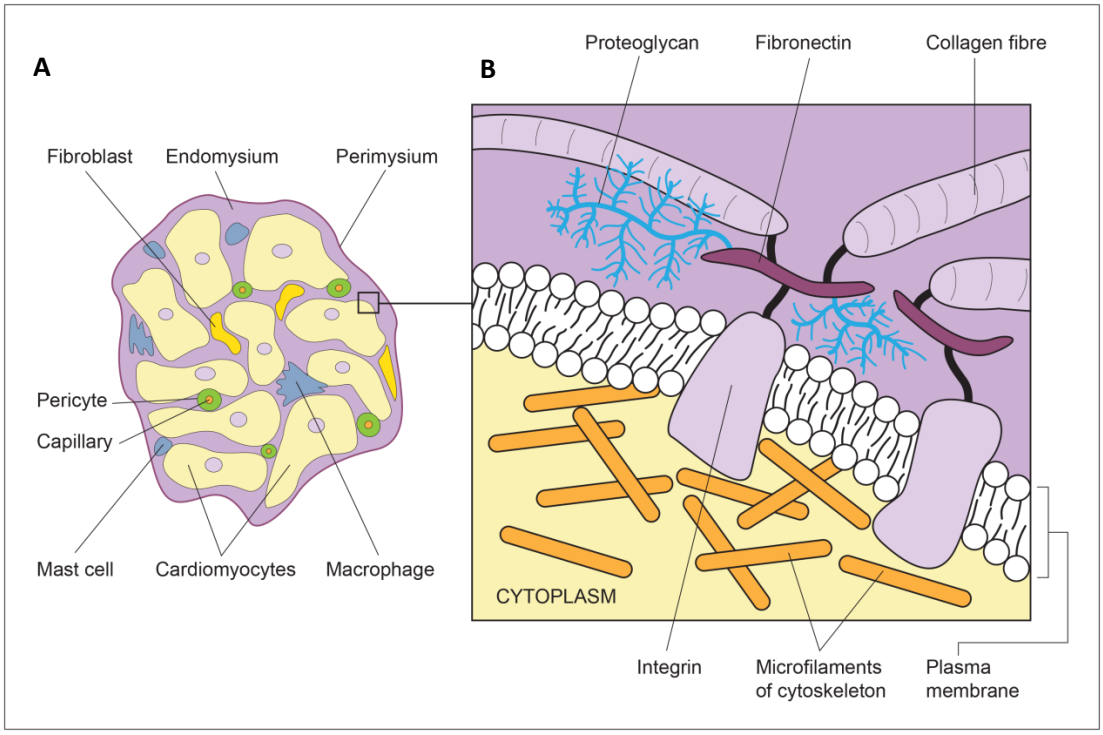


Figure 1.5 The Cardiac Extracellular Matrix. Cardiomyocytes arrange in a 'myocyte bundle' and the presence of the non-myocardial support cells and extracellular matrix **(A)** and a zoomed image of the cell membrane and the structures of the Cardiac ECM **(B).**

1.6.2 Extracellular Cardiac Matrix Function

The cardiac ECM facilitates enough force transduction to performed the mechanical work of the myocardium, alongside facilitation of intracellular communication and metabolism.[117] Structurally myocyte bundles and collagen fibres are uniquely distributed in the myocardium (**Figure 1.5**).[117] Myocyte bundles are grouped into sheets. The orientation of the myocyte bundles differs within the myocardium. The collagen fibres connect to individual myocytes and adjacent sheets.[117] **Figure 1.6** demonstrated the structural relationship of myocytes and collagen fibres in the LV.[117]

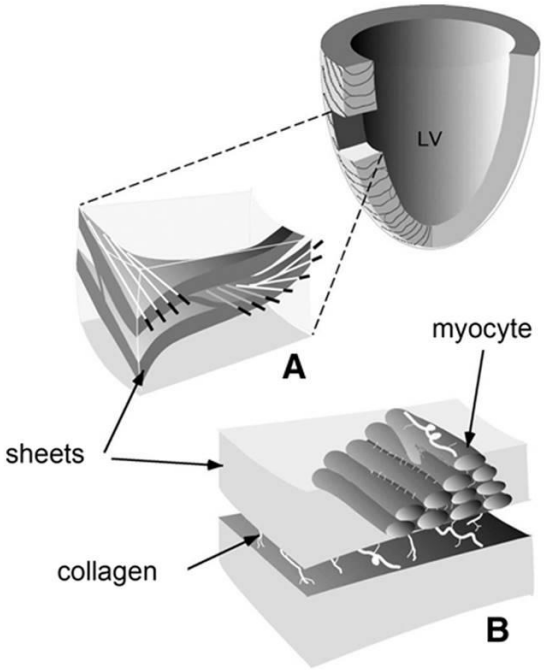


Figure 1.6 Normal 3-dimensional representation of cardiac microstructure. A representative transmural block taken from the ventricular wall (**A**) shows myofiber orientation which varies transmurally. Myocyte bundles are organized in sheets (**B**). Fibrillar collagens are seen interconnecting individual myocytes as well as adjacent sheets. Adapted from Ai-Hsien Li *et al*. Circ Res. 2014[117]

1.6.3 Cardiac Extracellular Matrix and Heart Failure

The cardiac ECM is a dynamic support structure that remodels following cardiac injury and HF.[94,113] ECM holds core roles in force transmission and alignment of myocardial fascicles (**Figure 1.6**) and provides substrate for the adhesion of myocytes.[113] Alteration in cardiac ECM turnover can impact cardiac structure and function directly. Loss of ECM can impact 000transduction of contractile force and change intracellular signalling of myocytes, which will change ventricular systolic function.[113] Accumulation of ECM can affect the myocardial passive stiffness properties, which will directly impact diastolic ventricular function.[113] In many pathophysiological situations following cardiac injury significant heterogeneity in ECM

remodelling can occur, where loss of ECM can also be accompanied by excessive accumulation leading to both ventricular systolic and diastolic impairment.[113] Cardiac ECM remodelling is closely related to adverse cardiac remodelling that occurs in HFrEF.[94,113] Progression remodelling is linked with severity and prognosis of HF.[94,113] Myocardial hypertrophy and overloaded hearts demonstrate alterations in collagen synthesis and degradation, which is linked to cardiac fibrosis and myocardial stiffness.[118-120] Development of cardiac fibrosis following ECM remodelling is linked to poor outcomes in HF.[121,122]

1.6.4 Collagen Turnover Biomarkers

Cardiac collagen turnover alterations are central to the development and progression of cardiac fibrosis and HF.[113] Collagenous cardiac ECM had been demonstrated to have a particular high turnover rate and this has been linked to diastolic stiffness.[115] Collagen turnover has the potential to generate biomarkers' of the pathophysiological ECM remodelling processes. Specific biomarkers of type I and type III collagen synthesis (N-terminal propeptides of type I and III procollagens (PINP and PIIINP)),[123,124] (carboxy-terminal propeptide of procollagen type I (PICP))[125,126] and degradation (carboxy-terminal telopeptide of type I collagen (ICTP or CITP))[119,126] products are associated with poor outcomes in HF. PINP and PICP markers have a stoichiometric 1:1 relationship upon deposition of type I collagen.[127] These markers offer the opportunity to examine cardiac ECM collagenous turnover in cardiac remodelling. Collagen turnover turnover biomarkers pose interesting predictors for CRT response. **Chapter 4** explores the current evidence through a systematic review to examine collagen biomarkers ability to predict CRT response in CHF.

There is a high amount of pre-analytical variation in collagen turnover biomarkers within and between subjects that should be accounted for and controlled where possible.[128,129] Collagen is an extracellular matrix protein found in all body tissues. Factors that particularly influence variability are age, gender, menopause, medication (e.g. anti-epileptics, bisphosphonates, steroids), fractures, bone pathology (osteoporosis), circadian rhythm, fasting and exercise.[128,129] The menopause has a large change on bone matrix turnover to the point that a different reference range is used for these patients.[128] Important controllable factors for collagen turnover biomarker variation are identified as the circadian rhythm, fasting and exercise. These factors should be accounted for study designs.[128,129] The other unmodifiable factors cannot be changed or planned around at a patient level and should be accounted for in the research design.

1.6.5 Matrix Metalloprotineases

The proteolytic enzyme system matrix metalloproteinases (MMPs) and their regulators tissue inhibitors of MMPs (TIMPs) are the main process by which collagen degradation is controlled. In normal physiological conditions, there is a balance between MMPs that degrade cardiac ECM components and TIMPs.[115] These proteases when an imbalance develops are linked to fibrotic diseases, cancer and cardiovascular disease[115,122,130,131]. In the heart the collagenase MMP-1 degrades structural collagens and gelatinases (MMP-2 and -9) degrade basement-membrane components and gelatins.[116] MMPs are secreted by a variety of cardiac cell types, including myocytes in an inactive form. Activation occurs by cleavage of the propeptide sequence, this can happen through several endogenous pathways including the urokinase/plasminogen cascade.[116] MMPs are also regulated at the transcriptional level,

by cytokines and growth factors. Neurohormonal systems have also been implicated as activators.[116] TIMPs are the counterbalance to this, the proteolytic enzyme system.[116] MMP activity (as opposed to expression level) has been linked to a large spectre of cardiovascular disease including atherosclerosis, aneurysms, myocarditis, hypertension and HF.[113,115,132,133] MMPs and TIMPs have been implicated in HF development and progression.[94,113] Specifically, MMP-1,[134] MMP-2[135] and MMP-9[136] and TIMP-1[134] are associated with HF outcomes. Alteration in MMP expression has been observed in small observational studies to reduce expression following CRT implantation in the short term.[132,137] MMPs and TIMPs pose interesting targets as biomarkers for CRT response predictors. **Chapter 2** explores the current evidence for MMPs and TIMPs ability to predict CRT response on CHF. Caution in interpretation must be demonstrated though at there are variations between the levels of expression between the literature[113,132,134] and other conditions can affect vascular MMP expression.[130]

1.6.6 Galnectin-3

Galectin-3 (Gal-3) is a beta-galactoside-binding lectin released by activated cardiac macrophages, which are up-regulated in HF, causing increased fibroblast proliferation, collagen deposition and ventricular dysfunction.[138] Gal-3' is strongly associated with inflammation and fibrosis with raised levels strongly predict poor HF outcomes.[138]

3.1 MiRNA

Micro Ribonucleic Acids (MiRNA) are short endogenous non-coding ribonucleic acids which are typically 20-22 nucleotides in length and there are in excess of 2000 in humans.[139,140] MiRNA were discovered in 1993 and found in humans at the turn of the century.[139] **Figure 1.7** schematically describes the transcription of miRNA from the genome into mature miRNA that regulate protein expression at the post transcriptional level.[139] Mature miRNA at the end of the transcription process enter RNA-induced silencing complexes (RISC) by associating with Argonaute proteins.[139,141] MiRNA inhibit protein translation and/or promote messenger ribonucleic acids (mRNA) degradation by specific targeting of the RISC to mRNA(s) (**Figure 1.8**).[142] Importantly, miRNA are now being recognised as key regulators of complex biological systems.[139] Fundamentally this is due to a single miRNA having the ability to regulate the expression of several genes.[139] This characteristic is dependent on the specificity of the target sequence.[139] Conversely, individual genes can be regulated by multiple miRNAs.[139]

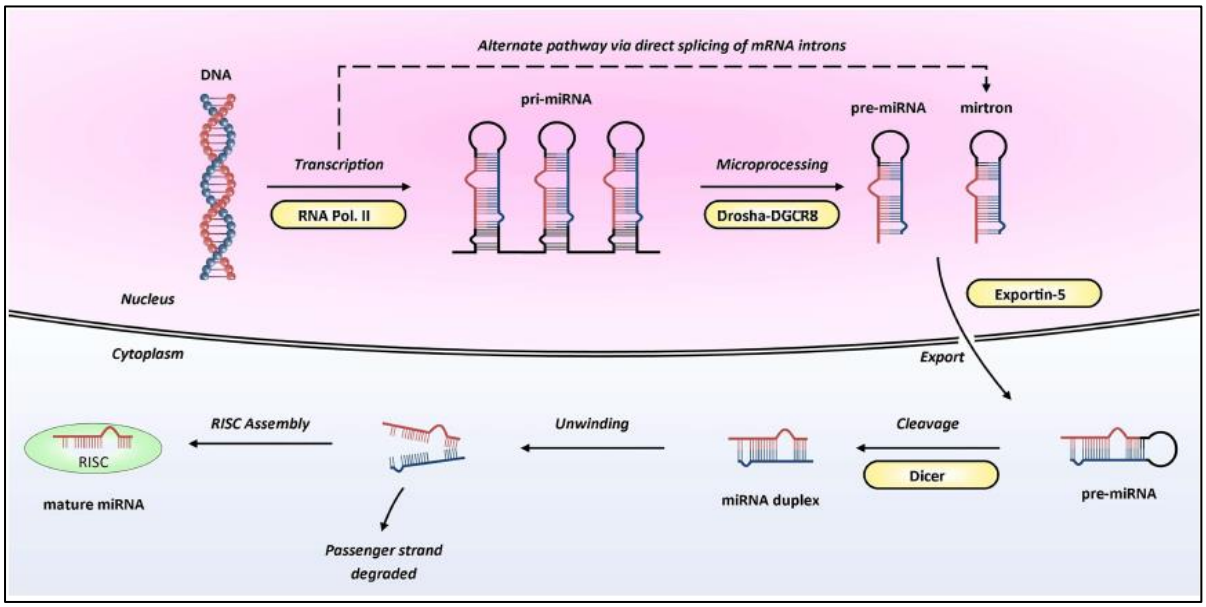


Figure 1.7 Biogenesis of miRNA. The miRNA genes are transcribed by RNA polymerase II (RNA-Pol-II) into molecules called primary miRNAs (pri-miRNAs). Inside the nucleus, these are cleaved into precursor miRNAs (pre-miRNAs) by an RNase III enzyme (Drosha), in conjunction with RNA-binding protein (DGCR8). Pre-miRNAs have a hairpin structure and are approximately 60–100 nucleotides in length. An alternative processing pathway of miRNAs exists, which bypasses the Drosha step and involves direct splicing of introns to create pre-miRNAs (represented by the dashed arrow), these are known as mirtrons. Both pre-miRNAs and mirtrons are actively transported to the cytoplasm by the Ran-GTP dependent transporter (Exportin 5). Once in the cytoplasm, pre-miRNAs are cleaved by RNase III (Dicer) generating a miRNA duplex (20-22 nucleotides) consisting of a functional (guide) and passenger. Dicer initiates formation of the RISC, involving the unwinding of the miRNA duplex and shedding of the passenger strand which is degraded. The RISC contains the mature miRNA, which can target mRNA. Depending on the extent of complementarity, the mRNA either gets degraded, or its translation is repressed. Taken from Romaine S *et al*. Heart 2015[139]

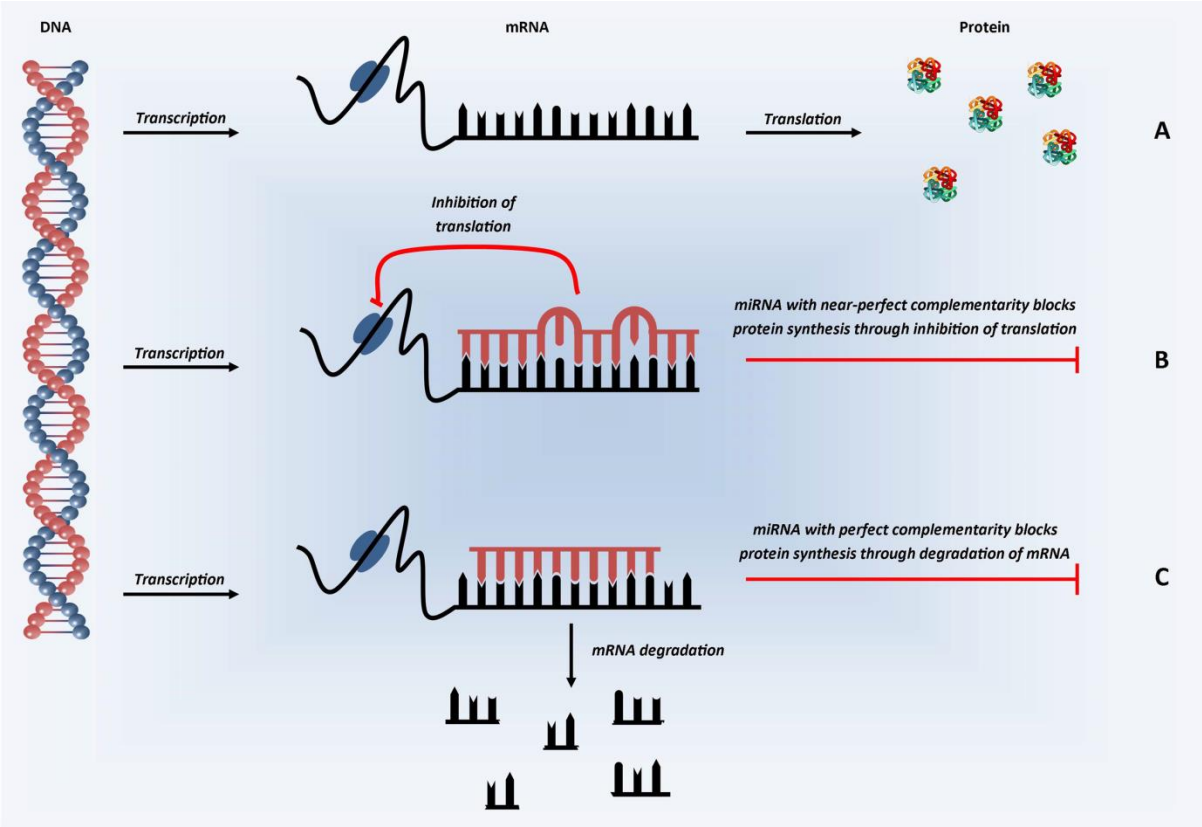


Figure 1.8 MiRNA Mechanisms of Action . Synthesis of a specific protein starts with mRNA being transcribed from DNA (transcription). When no miRNA is present, the mRNA transcripts are converted into protein (translation) (**A**). When miRNA with partial or near-perfect complementarity to the mRNA binds to the 30 untranslated region (UTR) it represses translation, which inhibits protein synthesis (**B**). When miRNA with perfect complementarity to the mRNA binds to the 30 UTR, it inhibits protein synthesis by inducing of mRNA degradation (**C**). In humans, perfect complementarity is rare, with varying degrees of partial complementarity the predominant situation. Taken from Romaine S *et al*. Heart 2015[139]

1.8 CIRCULATING MiRNA

Mitchell *et al,*[143] in their seminal paper, observed endogenous miRNA were present in human plasma and remarkably stable in the circulation. Stability was demonstrated by Mitchell *et al*[143] at room temperature and repeated freeze-thaw cycles. Resistance of circulating endogenous miRNA to RNAase activity was demonstrated, compared to exogenous synthetic miRNA that were degraded rapidly.[144] Circulating 'naked' miRNA are susceptible to rapid degradation, however miRNA residing in microvesicles (exosomes, microparticles and apoptotic bodies) remain protected.[141,144] A non-vesicle source of circulating miRNA is suggested following their detection after microvesicles isolation and subsequent high-speed centrifugation of plasma.[141,145,146] MiRNAs are also highly expressed in platelets.[147]

Circulating miRNA's represent exciting potential biomarkers for disease, given the limitations of other circulating biomarkers, especially the intra and inter individual variability. MiRNA are ideal biomarkers as they are easily accessible, relatively stable and in many instances tissue specific.[141] Circulating miRNA offer new potential biomarkers that may increase diagnostic yield, strengthen current risk stratification and deepen understanding of current biological/pathological processes.

1.9 MiRNA AND CARDIOVASCULAR DISEASE

MiRNA have emerged as having important mechanisms in human diseases including cardiovascular disease, diabetes and cancer.[142] Circulating miRNA have been observed to have altered expression in many different cardiovascular conditions compared to healthy patients.[148-151] Van Rooij *et al*[152] was the first to observe differentially expressed miRNA in

cardiac hypertrophy and HF in mouse models. Van Rooij *et al*[152] amongst the altered expression identified miR-195 was shown to promote cardiomyocyte hypertrophy and lead to HF.

Atherosclerosis is a multifaceted and complex pathology that is associated with multiple miRNAs altering expression.[139] Specific miRNAs have been shown to be associated with this process; miR-126-5p is associated with endothelial cell dysfunction due to reduced proliferation reserve promoting plaque formation,[153] and miR-143/145 complex is key regulator of vascular smooth muscle differentiation and is implicated in plaque formation.[154]

MiRNA differential expression has been observed in patients undergoing acute coronary syndrome events. MiR-1,[155,156] miR-208, [157] miR-133a[155] and miR-499[158] have been shown to be elevated in patients with acute MIs. Devaux *et al*[159] in a large (n=1,155) multicentre prospective observational study of unselected acute chest pain patients attending hospital identified significantly higher expression of miR-208b, miR-499 and miR-320a in those having acute MIs.[159] However, no miRNA demonstrated diagnostic superiority over cardiac troponin or high sensitivity troponin (hs-TnT). Zampetaki *et al*[160] identified in a large prospective observational study (n=826) that differential co-expression of endothelium-enriched miR-126 was associated with long-term risk of MIs.

1.10 MiRNA AND CARDIAC REMODELLING

Cardiac remodelling allows the heart to adapt to adverse stresses and injury. These adaption mechanisms allow remodelling to occur, which preserves cardiac output. These mechaisms

include but are not limited to; neurohormonal activation, pro-inflammatory changes, ECM turnover alteration and angiogenesis.[139,161] Chronic activation of these molecular mechanisms can become maladaptation and contribute to the development and progression of adverse cardiac remodelling.[161,162] MiRNAs hold a critical role in cardiovascular complex regulatory systems. Many different miRNAs are involved in regulatory roles of different molecular processes of cardiac homeostasis. Dysregulation of specific miRNAs have being observed in several cardiovascular conditions (as previously discussed). These processes when chronically activated aide the development and progression of HF.[139] Specific miRNAs dysregulation has been implicated in the alteration of these molecular mechanisms and contribute to the pathological development of HF.[161]

1.10.1 MiR-1

MiR-1 was the first miRNA discovered in a mouse heart.[163] It accounts for approximately 40% of all miRNA transcripts in the mouse cell.[163,164] MiR-1 has been demonstrated to be predominantly expressed by cardiomyocytes.[165] Sayed *et al*[166] was the first to identify a role for miR-1 in cardiac hypertrophy, observing in a mouse transverse aortic constriction model that it was down-regulated. Several targets have been shown to be down-regulated by miR-1; MEF2a, calmoudulin, GATA4, insulin-like growth factor and twinfillin, which causes cardiac hypertrophy.[164,167-169] Recently it has been demonstrated that adenoviral delivery of miR-1 to mice with transverse aortic constriction actually reversed hypertrophy, improved fractional shortening, reversed LV dilation and decreased fibrosis.[164,170] Mechanistically lower miR-1 expression has been observed to be associated with the development of cardiac hypertrophy.

1.10.2 MiR-21

Several miRNAs have been implicated in the regulation and development of fibrosis [161] MiR-21 is highly expressed in cardiac fibroblasts compared to other cardiac specific cells in the failing heart of both humans and mice. [161,171] Roy *et al*[172] first observed the miR-21 was upregulated in murine hearts in the infarct zone following ischaemia-reperfusion models. MiR-21 regulates fibrobast proliferation and survival.[161] MiR-21 levels decrease apoptosis through inhibiting *sprouty homolog-1* (SPRY1), which subsequently activates the ECM signal-related kinase mitogen-activated protein kinase (ERK-MAPK) enhancing the survival of cardiac fibroblasts.[171] This mechanism contributes to the induction of cardiac hypertrophy. These reports are supported by observations in a study where cholesterol-conjugated inhibitor of miR-21 (antagomiR-21) was administered in-vivo to mice models of cardiac hypertrophy (transverse aortic constriction or administration of isoproterenol) and found it limited the fibrotic response and improved overall cardiac function.

Recently in humans, the relevance of miR-21 was demonstrated in aortic stenosis patients undergoing surgical correction showing that levels were raised before but not after surgery and levels correlated with myocardial collagen.[173] However, the exact action of miR-21 remains unclear with opposing observations for the role and function of miR-21 being reported.[174] MiR-21 expression has been observed to decrease following an MI and over expression can reduce the size of the infarct.[175] MiR-21 is one of the most cardiac abundant miRNAs and demonstrates dysregulation in association with the development and progression of cardiac fibrosis. However, the exact behaviour remains unclear and there is limited information available on its clinical utility.

1.10.3 MiR-29

The miR-29 family are secreted selectively by cardiac fibroblasts and target mRNAs involved in extracellular matrix deposition.[161] In a mouse model of an induced MI, down-regulation of miR-29 was observed within the border zone, compared to the non-infarcted areas in the same heart.[176] MiR-29 has been demonstrated to target genes of proteins involved in remodelling and fibrosis including elastin, fibrillin-1, collagens type I and III.[176] Down-regulation of miR29 is observed to induce the fibrosis processes.[176] Introduction of anti-miRs in vitro and in vivo induces the expression of collagens, whereas over-expression of miR-29 in fibroblasts reduces collagen expression.[176] MiR-29 has also been implicated in myoblast transdifferation into myofibroblasts, matching its role to tissue fibrosis.[177] The evidence suggests that miR-29 has a central role in the regulation of cardiac fibrosis and downregulation can exhibit deleterious effects.[176]

1.10.4 MiR-122

MiR-122 is thought to be liver specific and has been shown to be involved in glucose and lipid metabolism.[178] Moreover, miR-122 has previously been demonstrated to be less abundant in the myocardium than the liver.[179] The role of miR-122 in the myocardium has been shown recently to have a potentially important role in cardiac fibrosis regulation. Beaumont *et al*[180] demonstrated miR-122 is down-regulated in myocardial biopsies from patients (n=28) with severe aortic stenosis. Futhermore, Beaumont *et al*[180] demonstrated that miR-122 down-regulation is associated with over-expression of transforming growth factor-ß1 (TGF-ß1), which leads to extracellular synthesis and deposition of type I collagen (producing PINP and PICP). TGF-ß1 is considered as the cardiac fibrosis master switch and

had been observed to be regulated directly by miR-133 and -590.[180] MiR-122 may have an important role in cardiac fibrosis and cardiac remodelling. The association seen in aortic stenosis has not been observed in other cardiovascular conditions. Moreover caution with interpretation of miR-122 should be taken as it is specific to the liver.

1.10.5 MiR-133

MiR-133 is one the most abundant circulating miRNAs in the human myocardium [161,164] There are 3 related miRNA; miR-133a-1,-133a-2,-133b. Mechanistically miR-133 may supress connective tissue growth factor, which is an important protein in the development of fibrosis.[181] The critical role of miR-133 in the development of cardiac fibrosis was demonstrated by the deletion of miR-133a-1 and -133a-2 in knockout mice leading to severe fibrosis.[182] Further mechanistic studies have also demonstrated that higher expression of miR-133 is cardioprotective against the development and progression of fibrosis.[161] Lower expression of miR-133 appears to have a role in the development of cardiac fibrosis.

MiR-133 has also been described as being associated in the development of cardiac hypertrophy. [183] Down regulation of miR-133 has been observed in cardiac hypertrophy murine models (transverse aortic constriction).[183] The down regulation of miR-133 was observed to correlate with increased LV wall stress.[183] MiR-133 has also be shown to target central regulators of cardiac hypertrophy (NFATc-4 and calcineurin)[184] and prohypertrophic mitogen-activated protein kinase pathways.[175]These results suggest that downregulation of miR-133 may be harmful and overexpression may offer cardioprotection.[164] The relationship of expression of miR-133 is far more complicated and tends to be related to the exact

context of the dysregulation to the exact phenotypic implications. In murine models it often depends on the exact cardiac hypertrophy induction method utilised.[164]

1.10.6 MiR-210

MiR-210 is known to have a pro-angiogenic function on cardiomycytes, stimulating the release of leptin, interleukin-1-a and tumour necrosis factor alpha.[161,185] Mechanistically miR-210 was observed when over-expressed in a mouse model (induced MI) to increase capillary formation, decrease apoptosis and minimise the size of the infarct.[161] MiR-210 is also known to be induced in hypoxic conditions, specifically by hypoxia-inducible factor 1α and cause angiogenesis.[186] Interestingly, on this basis, miR-210 has been theorised as a biomarker for HF. A recent observation was made that supported this hypothesis and that there was a negative correlation between high BNP levels and lower expression of miR-210.[149]

1.11 MiRNA AND HEART FAILURE

Differential expression of miRNAs has been repeatedly demonstrated in HF.[139] **Figure 1.9** summaries the circulating miRNAs that have been found to be differentially expressed in HF, the double border boxes demonstrated miRNAs that have had replicated in separate studies. Many of the studies have been performed on smaller cohorts and with varying quantification techniques.[139]

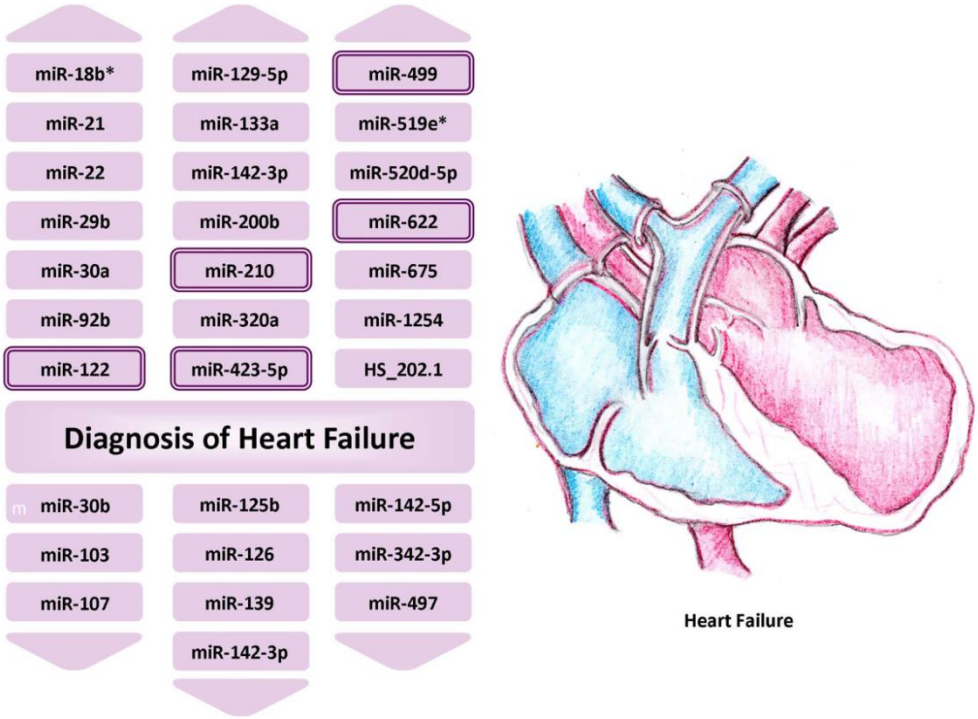


Figure 1.9 Circulating miRNA associated with Heart Failure. Taken from Romaine S *et al*. Heart 2015[139]

HF was initially explored as part of wider miRNA cardiovascular studies. Corsten *et al*[158] performed a small parallel case-control cross-sectional observation studies for four cardiovascular disease conditions (acute MI, viral myocarditis, diastolic dysfunction, acute HF) and examined a panel of miRNA tailored to each specific condition, using real time polymerase chain reaction (PCR). In the acute HF study 33 patients were compared to 34 healthy controls (not-matched) and had expression measured for six candidate circulating miRNAs (miR-1, -122, -133a, -208b, -223, and -499).[158] In the acute HF patients, miR-122 ($p<0.05$) and miR-499 ($p<0.05$) were found to have significant up-regulation compared to healthy controls.[158] MiR-122 is liver-specific and correlates with hepatic damage,[187] with the up-regulation likely being related to venous congestion.[158] This was a small explorative study, which was hypothesis generating only. The study undertakes several analyses in an

unmatched study design. Moreover the acute HF definition was based upon NT-*pro*-BNP level only, no account of cardiac structure or function was undertaken.[158] This study has limited value beyond hypothesis generation.

Studies recently have started to focus exclusively on HF cohorts to analyse different circulating miRNA expression patterns and whether they may have clinical value. In one of the earliest HF and miRNA studies Tijsen *et al*[188] performed a small discovery and validation study. The discovery phase analysed miRNA expression between 12 healthy controls and 12 patients admitted to hospital with acute HF. A microarray quantification approach was utilised and they identified 108 differentially expressed miRNAs.[188] Sixteen miRNA with the largest fold-change were selected to be tested in the validation phase, in three distinct patient groups (30 HF, 20 breathless without HF and 39 healthy controls) using real time PCR.[188] Seven miRNAs (miR-18b*,-129–5p,-423-5p,-622,-675,-1254 and HS_202.1) were observed to be significantly up-regulated in the HF group compared to the healthy controls, however only miR-18b* and miR-423-5p had significant up-regulation when compared to the non-HF dyspnoea group.[188] Only miR-423-5p was found to be a significant predictor in a multivariable logistic regression model of acute HF.[188] Mir-423-5p also strongly correlated with NT-*pro*-BNP and LVEF.[164,188] Although a potentially useful observation, the study has limited clinical value. First the study should only be considered exploratory given its size (HF n=42).[188] Secondly the HF characteristics make generalising these results difficult, the HF group included reduced and preserved ejection fraction patients, but only those with non-ischaemic aetiology. Thirdly a high proportion of the HF group study group were undertreated with OMT.[188]

Fan *et al*[189] recently undertook a small cross-sectional study of 45 dilated cardiomyopathy patients and 39 sex-matched controls to examine the differential expression of five candidate miRNAs (miR-126,-146a,-155,-361-5p and-423-5p). MiR-423-5p was demonstrated to have higher expression in dilated cardiomyopathy patients (p=0.003) and a positive correlation with NT-*pro*-BNP (r=0.430, p=0.003).[189] Furthermore miR-423-5p demonstrated a moderate ability to discriminate between dilated cardiomyopathy patients and healthy controls (area under the curve (AUC) ROC 0.67).[189] The results from Fan *et al*[189] support the initial findings from Tijsen *et al*,[188] however this was only a small exploratory study. Bauters *et al*[190] in a larger prospective observational study (n=246) of patients with anterior MIs. Candidate miRNAs (miR-133a and -423-5p) were compared to recorded echocardiography and NT-*pro*-BNP over 12 months.[190] It was observed miR-133a and miR-423-5p were significantly raised following an anterior MI over the next year but these findings of Bauters *et al*[190] did not correlate with LV geometric features or NT-*pro*-BNP.[190] Bauters *et al*[190] conducted a larger clinical study, which allows more weight to be put upon its findings, however the results cannot be compared to Tijsen *et al*[188]and Fan *et al*[189] due to the different aetiologies of HF involved.

More recently Vogel *et al*[191] undertook a case-control study with a discovery (microarray) and a subsequent validation experiment (real time PCR) examining miRNA differential expression in non-ischaemic HFrEF patients compared to healthy controls. MiR-200b, miR-622, and miR-1228 demonstrated significant up-regulation in HFrEF patients in both experiments. [191] Interestingly Vogel *et al*[191] demonstrated miR423-5p was up-regulated but

did not reach statistical significance, contradicting Tijsen *et al*.[188] Vogel *et al*[191] also demonstrated alteration in expression of circulating miRNA within serum and different cell types. This article goes some way to overcoming the limitations of Tijsen *et al*.[188] focusing on a specific group of HF patients, minimising heterogeneity in the study cohort.[191] The study however should still be considered as explorative as the cohort is small (HF n=53).[191]

Circulating miR-210 is known to be induced by hypoxia and theoretically was queried to be biomarkers for HF. Endo *et al*[149] observed in an animal model that Dahl rats with HF had up-regulation of miR-210 in mononuclear cells and skeletal muscle. MiR-210 was examined in two small human cohorts with HF (classified by NYHA only); one cohort had mononuclear cell derived miR-210 expression compared between 13 HF and 6 healthy controls, which showed significant up-regulation in NYHA III/IV patients compared to controls/NYHA II. The second cohort involved 39 HF patients having plasma samples taken and 24 of these patients having repeat samples taken at least 3 months after and this observation showing there was no correlation with BNP, but those patients that improved (lowering BNP) had lower initial miR-210.[149] The study is a translational study that highlights miR-210 as a potential biomarker for HF, however very limited clinical information can be provided by this study.

Recently, Goren *et al*[192] in a case-control cross sectional study analysed 186 circulating miRNAs in 30 HFrEF (LVEF<40% without coronary disease) against 30 age-sex matched controls. Goren *et al*[192] demonstrated miR-423-5p, miR-320a, miR-22, and miR-92b had significant up-regulation compared to controls and a combination score of these 4 miRNAs

could discriminant non-ischaemia HF cases. However, this is the first and only time this score has been applied to a very small cohort, it requires validation. Furthermore the majority of the HF cohort were on OMT and 53% had a complex cardiac device. [192] None of the controls had a complex cardiac device or were on OMT.[192] Up-regulation could be related to these confounding treatment.[192] These results should are exploratory and therefore of limited clinical value. However the miR-423-5p observation replicates the Tijsen *et al*[188]and Fan *et al*[189] findings.

MiRNAs have also recently been examined as to whether specific miRNA expression signatures can differentiate HFrEF from HF with preserved ejection fraction (HFpEF). Two studies in 2015 examined this question and provided contrasting answers. Watson *et al*[193] undertook a discovery and validation study on three groups of patients (90 HFrEF, 90 HFpEF, 90 controls). The discovery phase quantified 745 miRNAs with a microarray in 15 pooled plasma samples from each cohort.[193] Five miRNA (miR-30c, −146a, −221, −328, and −375) demonstrated differential expression between both HF vs controls and HFrEF vs HFpEF.[193] The validation phase specifically quantified those five miRNAs with PCR in the remaining participants from each group.[193] These five circulating miRNAs showed no greater discriminatory power than BNP for HF or non-HF.[193] MiR-375 showed the greatest ability to differentiate HFrEF vs HFpEF.[193] A prediction model including BNP, miR-30c, miR-221, miR-328, and miR-375, produced the greatest ability to differentiate HFrEF vs HFpEF (AUC 0.854).[193] In contrast Wong *et al*[194] identified different miRNAs expression patterns between HF vs non-HF and HFrEF vs HFpEF. Wong *et al*[194] studied three small groups (69 HFrEF,49 HFpEF and 58 non-HF) in a cross sectional discovery and validation study to examine for

differences in miRNA expression profiles.[194] A microarray was used to screen miRNAs in the discovery phase and then specific miRNAs were validated with quantitative PCR.[194] Wong *et al*[194]screened 806 miRNAs in the discovery phase, of which 90 had statistical significant different expression to meet criteria to be tested in the validation phase.[194] Twelve miRNAs (miR-125a-5p, -183-3p, -190a, -193b-3p, -193-5p, -211-5p, -494, -545–5p,-550a-5p, -638, -671–5p and -1233) were found to have significant differential expression between HF vs non-HF controls and/or HFREF vs HFPEF in both blood and plasma.[194] MiR-125a-5p, -190a, -550a-5p and -638 were significantly different between HFrEF vs HFpEF.[194] Moreover miR-183-3p, -190a, -193b-3p, -193b-5p,-211-5p, -494, -671-5p, and -1233 were found to differentiate between HF vs non-HF.[194] MiRNA panels for HF and HFrEF were identified as having discrimination power greater than NT-*pro*-BNP.[194] Interestingly Wong *et al*[194] did not observe any difference in expression for miR-423-5p between HF vs non-HF. A criticism of Watson *et al*[193] in the literature was that only one reading was taken from pooled samples in each cohort on the microarray, likely leading to some miRNA not being detected and outliers of others affecting the recorded expression.[195] This may partly account for the complete contrast in miRNA observed to be predictors in the two studies.[193,194]

Heart Failure and HFrEF in particular has had a variety of differential miRNA signatures demonstrated to healthy patients. In many studies these miRNA either individually or as part of a panel have demonstrated the ability to diagnose or prognosticate. The challenge remains that these are often small discovery and validation studies that cannot be applied clinically. They often demonstrate heterogeneity between cohorts or mRNA analytical techniques. MiRNA biomarkers are often not replicated consistently. MiRNA has an

important role in the development of adverse cardiac remodelling in HF, but a specific biomarker has not yet been validated.

1.12 MiRNA AND CARDIAC RESYNCHRONISATION THERAPY

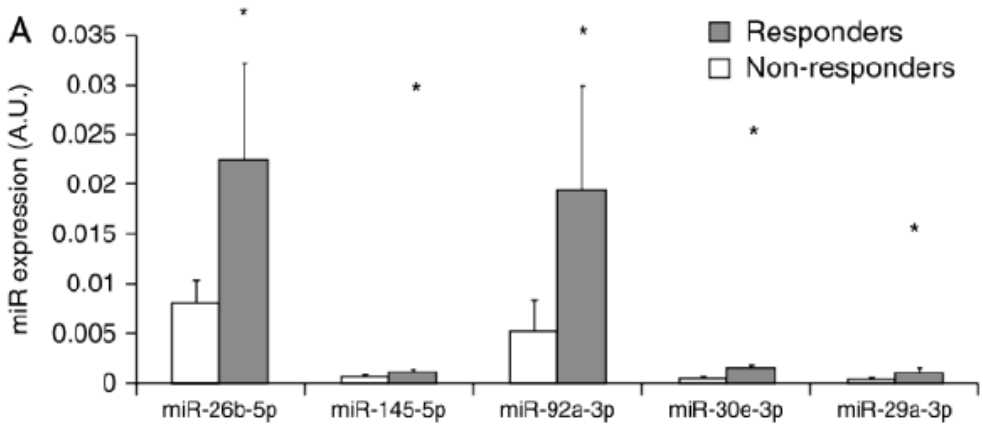
Multiple miRNAs have been observed to be dysregulated and implicated in the development and progression of HF. The evidence remains limited due to the principle research being basic science and small cross section cohort studies. Based upon observations, the hypothesis was generated that miRNAs might be able to predict CRT response and outcomes.

Recently Ning *et al*[196] performed the first study examining miRNA and CRT. Ning *et al*[196] undertook an animal study comparing four groups of rabbits (10 in each group) and induced HF in three groups (by ascending aorta cerclage constriction). Two groups were paced with biventricular or LV alone; the third group had a sham procedure.[196] Twelve weeks after the procedure had been performed pacing was performed for six hours a day for seven days. Following this period of pacing LVIDD decreased and LVEF increased significantly.[196] Plasma was taken to measure circulating miR-133 and was found to have significantly lower expression in the HF sham group compared to the control. Both pacing groups had significantly higher expression than HF sham group. The biventricular pacing group had the greatest rise in expression ($p<0.05$). These findings support the observations miR-133 is cardioprotective in HF.[161,181,182] Ning *et al*[196] performed an animal study and therefore it can only be hypothesis generating; miR-133 up-regulation is increased with biventricular pacing.[196]

Simultaneously Marfella *et al*[197] published their prospective observational, non-randomised, self-control study on miRNA expression in HFrEF patients before and after CRT implantations. All consecutive patients (NYHA III/IV, sinus rhythm QRS≥120msec, OMT≥6 months and LVEF≤35%) scheduled for CRT (153 patients) between January 2009 and August 2011 were screened and 81 subjects (HF cohort) were recruited in three Italian centres.[197] A test (15 healthy volunteers) and validation (60 controls - age, sex and co-morbidity matched) cohorts were recruited to compared HF miRNA expression too.[197] The use of a healthy control cohort is an interesting one as they are not matched in any way to the HF group. The HF cohort underwent functional (6MWT) and transthoracic echocardiography assessment at baseline and 12 months follow-up.[197] The 6MWT performed was not performed in the standardised fashion as per the American Thoracic Society guidance.[102] CRT response status was defined by degree of LV reverse remodelling (↓≥10% LVESVi and ↑>10% LVEF and no heart transplant).[197] There were 55 (67.9%) responders and 26 (32.1%) non-responders. [197] There were four mortalities in the observation period that were excluded following CRT implantation, inferring a bias towards selecting healthier HF patients and not accounting for those that had died. [197]

A microarray was utilised to screen 84 miRNA pre-selected for their reported association with structural heart disease.[197] Marfella *et al*[197] observed the HF group had 49 down-regulated miRNAs compared to healthy controls and 24 miRNAs compared to the validation cohort (all $p<0.05$).[197] Baseline expression of five circulating miRNA's (miR-26b-5p,-29a-3p,-

30e-5p,-92a-3p and -145-5p) in HF patients directly and indirectly correlated with LVEF and NT-*pro*-BNP.[197] Furthermore these 5 miRNAs demonstrated a high AUC (>0.83) in differentiating HF cases from non-HF.[197] Baseline expression of all circulating miRNA showed no statistically significant difference between RvsNR.[197] Following one year, responders had 19 miRNAs significantly (p<0.01) up-regulated (15 miRNA had >5-fold-change increase) and non-responders had 6 miRNAs significantly up-regulated from baseline (from the 24 identified from comparison with the validation cohort).[197] **Figure 1.10** shows that five miRNAs all showed up-regulation regardless of response status, however it was significantly higher (p<0.001) for responders.[197] All these five miRNA have known mechanistic roles in maladaptive molecular processes; hypertrophy (miR-26b-5p and -30e-5p), fibrosis (miR-29a-3p and miR-92a) and apoptosis (miR-145).[197] Interestingly miR-885-5p was observed to be up-regulated in non-responders, but no change in responders.[197] The changes in expression over 1 year for these five miRNA correlate with LVEF and NT-*pro*-BNP.[197]



B

	miRNAs changes 1 years after CRT			
hsa-miRNA	Fold Regulation vs baseline		P-value vs baseline	
	Responders	Non-responders	Responders	Non-responders
miR-26b-5p	15.2±2.8	5.6±2.4	0.002	0.0319
miR-145-5p	6.2±1.1	2.4±1.2	0.000	0.0402
miR-92a-3p	13.6±1.9	3.6±1.5	0.003	0.0241
miR-30e-5p	7.8±1.4	2.1±0.9	0.000	0.046
miR-29a-3p	9.0±1.3	2.5±1.3	0.012	0.162

Figure 1.10 MiRNA Expression Profiles for Responders vs. Non-Responders after one year of CRT. MiRNA listed are those that demonstrated a significant difference between RvsNR in fold change over one year observation. **Panel A** gives miRNA expression (arbitrary units=AU) after one year of CRT. **Panel B** provides a Table describing the degree of miRNA fold regulation from baseline to one year following CRT and its specific significance. *p<0.01 responders vs non-responders. Taken from Marfella *et al.* EHJ. *2013*[197].

Marfella *et al*[197] examined several miRNAs involved in regulated maladaptive molecular processes implicated in adverse cardiac remodelling in HFrEF. Marfella *et al*[197] demonstrated altered expression of important miRNAs known to be implicated in several maladaptive processes in HF and furthermore demonstrated an association with LV echocardiographic and neurohormonal variables that are known to improve following CRT implantation. Moreover, variation is seen between responders and non-responders at

follow-up identifying alteration in the molecular processes. However, no difference between these miRNAs were demonstrated between responders and non-responders baseline (pre-implant) levels, offering no predictive value for any of these biomarkers.

There are several limitations of Marfella *et al*[197] that need to be considered. Firstly this was a small cohort study that lacked power to support their observations. Secondly, the miRNA quantification method should be acknowledged as not being the gold standard. Thirdly, the healthy control comparison cohort was not age-sex matched, therefore the high number of miRNAs expression demonstrated is understandable and accountable by potentially multiple physiological processes.[198] These limitations do challenge the use of this paper in the wider literature to miRNAs as predictors of CRT response.

More recently Melman *et al*[199] moved the debate on about altered miRNA expression profiles in HFrEF patients undergoing CRT implantation with the publication of their translational study. Melman *et al*[199] performed an exploratory discovery and validation prospective cohort study on differential miRNA expression in 52 HFrEF patients referred for CRT implantation (NYHA II-IV, LVEF$\leq$35%, QRS$\geq$120msec and LBBB/RV paced) between responders and non-responders. Response was defined as an increase LVEF >10% at six months on transthoracic echocardiography for the validation phase.[199] A major limitation of the methodology is concerned with the performance of the transthoracic echocardiography; only a single plane was used to calculate LVEF, this does not meet the internationally accepted national standard[200] The standard is to perform Simpson's biplane assessment for

LV ejection fraction (**Chapter 5.5**).[201] Aside from not meeting the national echocardiography standard it makes comparisons between different studies that measure LV geometry unreliable.

The discovery phase was performed on 12 select patients; 6 responders with the greatest LVEF increase and 6 non-responders with no change/decline.[199] All the participants in the discovery phase were men with non-ischaemic cardiomyopathy and LBBB to increase the sensitivity of the screening.[199] A microarray was used to screen 766 human circulating miRNA in plasma, which identified four miRNAs (miR-30d,-99b,-409-3p and -766) had significantly differential expression between responders and non-responders (all $p<0.05$).[199] The validation study was performed in the remaining 40 participants with no significant characteristic differences between RvsNR.[199] Surprisingly only 58% of the total validation cohort were on Angiotensin Converting Enzyme Inhibitors (ACEi); most HF studies have HFrEF patients have on higher amounts of OMT. Furthermore, the validations cohort included 33% AF patients and 20% ischaemic cardiomyopathy, which made the Melman *et al*[199] validation cohort more heterogeneous than in the Marfella *et al*[197] HF cohort. However, it does question the selection of the four miRNAs they took forward into a validation cohort with different baseline characteristics. [199] The was a high proportion of the cohort on anti-platelets (83% Aspirin, 23% Clopidogrel) and anticoagulants (45% Coumadin), which are known to affect miRNA expression, especially platelet derived.[147,199] There were 21 (52.5%) responders and 19 (47.5%) non-responders.[199] Ten candidate miRNAs were quantified in the validation cohort with qPCR; 5 miRNAs were already identified and five miRNA (miR-18b,-29c,-129-5p,-423-5p and -622) not identified in the discovery phase. Four

miRNAs (miR-29c,-30d,-142-5p and -766) were significantly overexpressed in responders (all $p<0.05$).[199] Multivariable logistic regression (accounting for co-variates) identified higher circulating miR-30d (OR=2.52, CI (95%) 1.07-5.94) and miR-142-5p (OR=2.47, CI (95%) 1.26-4.85) predicted a better CRT response.[199] A multivariable linear regression model, for degree of change in LVEF over six months, identified only miR-30d as being significantly associated ($p=0.02$) with change in LVEF at six months.[199] **Figure 3.5** demonstrates baseline miR-30d significantly correlated with change in LVEF over six months (Þ=0.39, $p=0.01$). However the strength of correlation is driven by the two highest changes in miRNA expression which are treated as outliers, when these points are removed the significance of the correlation is reduced (Þ=0.31, $p=0.058$), a point Dorn[202] summaries in the sister editorial.

A separate 21 HFrEF participants formed a test cohort who had undergone a CRT implantation and had six months samples.[199] The test group was used to compare to the results of the validation cohort. The additional 21 baseline samples demonstrated the strength of miR-30d to predict CRT, supporting the validation cohort findings. The six month follow-up samples identified that miR-30d expression had decreased after six months following CRT implantation significantly in responders ($p=0.05$), but not in non-responders.[199]

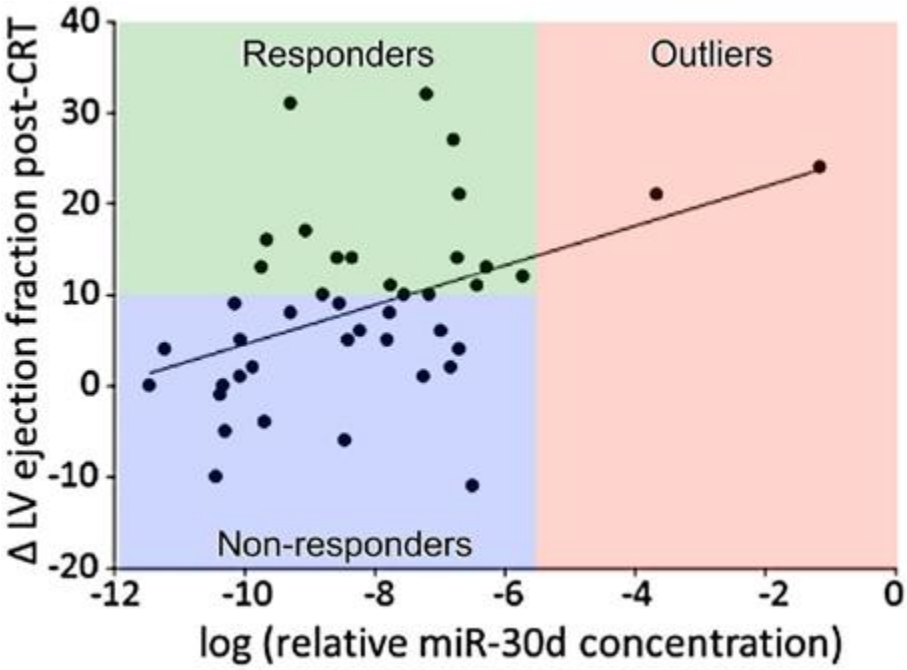


Figure 1.11 Relationship between circulating plasma miR-30d levels and change in LVEF six months after CRT. Correlation with all data points was significant (Þ=0.39, *p*=0.01), however when outliers removed correlation less significant (Þ=0.31, *p*=0.058). Red highlighting indicates miR-30d outliers, green are responders, blue are non-responders. Taken from Dorn EW. Circulation 2015.[202]

Biologically Melman *et al*[199] demonstrated that miR-30d was expressed in higher concentrations in the CS, synthesised and released from cardiomyocytes in extracellular vesicles.[199] Spatial heterogeneity in the LV of miR-30d was demonstrated in a canine dyssynchronous HF model, with the lateral wall showing higher expression.[199] Furthermore Melman *et al*[199] demonstrated that miR-30d expression and release from cardiomyocytes is associated with increased mechanical stress, mediating cardiac hypertrophy. MiR-30d was observed to protect against tumour necrosis factor-α (medicates apoptosis), inferring a

cardioprotective role.[199] Increased expression of miR30-d seems to represent a favourable adaptive process which may predict CRT response.

Interestingly following the publication of Melman *et al's*[199]miR-30d and CRT translation study[199] the authors of the first CRT and miRNA cohort expression paper[197] wrote a published response contrasting how miRNA expression profile was measured and the common themes emerging regarding the miR-30 family. Sardu *et al*[203] highlighted the differential miRNA expression pattern seen and that miR-30e were observed to be up-regulated at one year follow-up. They commented this might have potential anti-apoptosis effects. Structurally the miR-30 family is very similar and the biological function of members of the group is thought to be similar.[203] Melman *et al*[203] responded to these comments supporting the observation that other extracellular miRNA were likely to be implicated in the reverse LV remodelling. They also pointed out the differences in miRNA expression observed was likely to be due to small cohorts, differences in patient characteristics and the accepted variation in methodologies on quantifying miRNA expression. Importantly both authors agree on the importance of the miR-30 family in the process of reverse LV remodelling induction by CRT and the modification of the maladaptive molecular processes of apoptosis this regulates. Moreover the potential value in detecting response status and other extracellular miRNA are likely to be important in this regulation process.[203,204] Melman *et al*[199] has particularly demonstrated a striking difference between response status and the molecular mechanism it is involved in regulating. However the pattern of miR-30d was shown to contrast that reported by Marfella *et al*[197] as it showed it was not overly expressed at baseline and no changes occurred in responders, unlike Melman *et al.*[199] The translational

pilot offers a very robust assessment of the biological mechanism of the likely reason miR-30d is a potential biomarker of CRT response, further validation is required.

1.13 MiRNA DISCUSSION

The research in miRNA and cardiovascular disease has sunstantially increased over the last decade with the identification of their stability in the circulation.[143] There are patient specific issues that affect miRNA expression that need to be accounted for when assessing their potential as biomarkers. Moreover the methods available for quantification are varied and non-comparable amongst the literature where different methodologies are avaliable.[198] Heart Failure has identified that multiple miRNA are differentially expressed and may have the potential to diagnose and prognosticate the condition. However these studies are commonly small cohort studies, hence the variability in the results reported in the literature, resulting in most published articles being hypothesis generating only and lacking power to have any significant implications for patients. MiR-423-5p is an example of this hypothesis generation in the literature as a potential diagnostic biomarker[188] but the results are not consistently replicated.[191] MiRNA dysregulated in HF often reflects the development of maladaptive molecular processes that are known to be associated and important in the development of adverse cardiac remodelling. Cardiac devices are known to reverse remodel and modification of the differential expression patterns have been identified.[205] CRT recently has shown different miRNA expression patterns following implantation and variation dependent on the degree of reverse LV remodelling.[197] More recently a candidate miR-30d has been shown to alter in responders and be associated with a predictive response, due to over expression being demonstrated to be anti-apoptotic.[199]

Further exploration of dysregulated miRNA associated with maladaptive strategies would be potentially beneficial for identifying biomarkers of CRT response.

1.14 HEART FAILURE METABOLISM

Under normal physiological circumstances there is a balance between anabolic and catabolic metabolism and its regulation. The development and progression of HF is associated with neurohormonal systems activation, the development of a pro-inflammatory state and endothelial dysfunction.[2,206,207] The imbalance in metabolism that favours a pro-catabolic state is associated with progression of HF and alters skeletal and adipose tissue metabolism.[206]

Natriuretic peptides (e.g. BNP and NT-*pro*-BNP) are released in response to the haemodynamic changes in HF and convey diagnostic and prognostic value.[2] An inverse relationship is well established between natriuretic peptides and BMI.[208] Christensen *et al*[208] in a small cross-sectional study observed high NT-*pro*-BNP were associated with low total fat mass (ß=-0.3, $p<0.05$).

Adipocytes are sensitive to natriuretic peptides, activating lipolysis and enhancing the expression of brown adipocyte genes; increasing energy utilisation and thermogenesis.[206] Natriuretic peptides stimulate the release of adipokines, specifically adiponectin and leptin, which increase energy utilisation and weight reduction.[206] Adipokines are involved in whole-body energy metabolism, and adiponectin is particularly involved in the regulation of skeletal muscle metabolism and weight loss in HF.[209] Loncar *et al*[209] in a cross-sectional

study of elderly males with stable HF and no cardiac cachexia (CC) observed adiponectin was independently associated with muscle mass and strength. In a pivotal prospective observational study of RV dysfunction and CC (n=408), Melenovsky *et al*[207] identified adiponectin levels were significantly raised in participants with RV dysfunction and who were cachectic. Furthermore, adiponectin was one of the few variables (alongside NT-*pro*-BNP, RV dysfunction and of neurohormonal antagonist therapy) to independently predict CC.[207] Serum adiponectin is associated with severity of HF and adverse outcomes.[209] Paradoxically, adiponectin have been observed to have beneficial effects on lipid and glucose metabolism, alongside myocardial inflammation, hypertrophy and fibrosis.[210] It has been identified as a well-placed potential biomarker for the cross-talk in HF metabolism.[210]

Pro-inflammatory signals from cytokines and interlukin-6 (IL-6) are increased in HF.[206] Proteolysis in muscle occurs predominantly via the ubiquitin–proteasome system, which has increased activation HF due to stimulation from these increased pro-inflammatory signals.[211] The ubiquitin–proteasome system degrades proteins, the rate of which increases in HF.[211] Christensen *et al*[206] described a trend towards an association between high IL6 and Lean mass. Adiponectin and leptin have receptors in skeletal muscle that have acute and chronic effects on local metabolism.[209]

1.14.1. Adiposity and Heart Failure

Obesity defined as a raised BMI $\geq$30.0kg/m^2, is recognised as a risk factor for HF. There is a 5% and 7% increased risk of developing HF for every one unit rise in BMI, independent of important co-variables, for men and women respectively.[212] A graded increase in risk of

developing HF is recognised for increasing BMI in both males and females in different population groups.[212] Furthermore, Clark *et al*[212] describes the increased risk of HF from other raised adiposity surrogate metrics, for examples waist circumference and waist hip-ratio.

Counterintuitively, those with a raised BMI and established HF have been observed to have improved outcomes.[212-215] Oreopoulos *et al*[213] in a large meta-analysis of 28,209 HF patients who were obese or overweight (25.0-29.9 kg/m^2) showed a reduction in all-cause mortality (-19.0% and -40.0%) and cardiovascular mortlaity (-16.0% and -33.0%) respectively compared to those without a raised BMI ($\leq$24.9 kg/m^2) at >2years follow-up. The relationship between BMI and mortality has been demonstrated to have a U shaped curve with the lowest rates being associated with those overweight and obese and the higher rates being associated with leanness and severe obesity (>35.0 kg/m^2),[214] though not all datasets have replicated this finding.[215] The inverse relationship of NT-*pro*-BNP and adiponectin with BMI and total percentage body fat is suggestive that a higher fat content protects against the pro-catabolic activity of these neurohormonal signalling pathways.[208,209] Cai *et al*[216] demonstrated in a retrospective cohort study of Chinese patients with severe left ventricular systolic dysfunction (ejection fraction $\leq$35%) patients (n=219) that were overweight (24.0-28.0 kg/m^2) and obese (>28.0 kg/m^2) predicted Cardiac Resynchronisation Therapy response and improved survival at six months. Notably, within this study, the defined BMI ranges were lower than other studies due to the Chinese population having a lower average BMI than western populations.[216] Furthermore, this study demonstrated that

the obese population better tolerated optimal medical therapy, an observation noted in other studies.[207,216]

The significance of these paradoxical observations of the impact of adiposity are highly debated, does it represent a significant impact on metabolism in HF or is it a spurious observation from predominantly cross-sectional research articles? Several explanations and hypothesis have been offered to explain this phenomenon. Firstly lower natriuretic peptides are observed in obese patients and as a result symptoms may occur later. Secondly, the downgraded natriuretic peptide system is associated with adiposity and therefore offers a 'protective' role.[212] However the opposing antagonistic endogenous circulating renin-angiotensin-aldosterone system (RAAS) is dysregulated in patients with adiposity due to the development of a local RAAS system in visceral adipocytes and angiotensin acting as a growth factor for adipocytes.[217] The impact of the dysregulation of both systems with adiposity is not entirely clear. Thirdly, BMI is a crude metric of body composition, not accounting for proportions of each component, but is often used in studies.[212] Finally, obesity is a heterogeneous condition with various fat mass distributions, including; visceral fat deposits or subcutaneous/gluteofemoral obesity. Each have differing metabolic profiles.[212] The presence of established adiposity is observed to slow the progression of metabolism to a pro-catabolic state in HF, but the true impact is not clear yet[218]

1.14.2. Body Composition and Heart Failure

Muscle wastage is a common occurrence in HF.[211] Sarcopenia is defined as reduced muscle mass and limited mobility, occurs naturally with aging at of a rate of 1-2% per annum over

50 years of age.[211] Fülster *et al*[211] recruited 200 HF patients at a single centre and observed 19.5% of the cohort had sarcopenia, a higher proportion than expected through natural aging. Significantly both reduced (68.8%) and preserved (31.2%) ejection fraction HF patients were recruited to this study.[211] HF patients with sarcopenia had higher incidence of reduced left ventricular ejection fractions, reduced muscle strength, worse functional capacity and significantly higher IL-6 levels.[211] Elevated pro-inflammatory signals in HF, including cytokines and interlukin-6 stimulate catabolic pathways; for example the ubiquitin-protease pathway and cause sarcopenia.[211] Neurohormonal signalling cross-talk has been shown to be associated with muscle wasting.[209]

CC is defined as the unintentional non-oedematous $\geq$5% weight loss over $\geq$6 months, though higher weight loss levels have been set in the literature.[208,214] The imbalance of metabolic systems progressing towards a catabolic state in HF results in CC. One of the strongest and most reproducible poor prognostic signs in HF is development of CC.[207,212-214] Pocock *et al*[214] in a sub-study of the 'Weight loss and mortality risk in patients with chronic HF in the candesartan in HF: assessment of reduction in mortality and morbidity (CHARM) programme[12] (n=6933), demonstrated that together leanness ($\leq$22.5 kg/m^2) and CC (unintentional $\geq$5% weight loss at $\geq$6 months) increased mortality rate by 150% at 37.7 months. Melenovsky *et al*[207] observed CC with RV dysfunction (vs non-CC with a normal RV) was apredictor of adverse events in HF patients (HR 6.7, CI (95%) 4.1-10.9, p<0.0001).

The prevalence of CC has improved with the introduction of new treatments and now is estimated to be 10.5% in the stable HF patients.[208] CC represents wasting across all body

tissues though sarcopenia was initially described as being the critical trigger,[208] though more recently fat mass loss has been associated with CC.[207] Neurohormonal and inflammatory signals have also been shown to be elevated in CC and have their own prognostic value in HF.[206-208,212] CC represents the critical step in the pro-catabolic transition of the body's metabolism in advancing HF, though the cross-talk of signalling demonstrates a heterogeneous picture of triggers and regulation. Further understanding is required.

1.14.3. Conclusion

There is clear interplay in HF development and progression on metabolism and body composition. The literature in this field is limited to small cohort or cross sectional studies. The complexity of the interplay is clear and full understanding of development, regulation, progression and ultimately clinical value has yet to be achieved. The prognostic value of certain biomarkers is critical in predicting adverse outcomes. CRT implantation offers a good in-vivo model to see the impact of reverse cardiac remodelling on body composition and the potential clearer understanding of the interplay.

1.15 PUBLICATIONS

*Section 1.2 was published as a review article entitled 'Cardiac Resynchronisation Therapy and its role in Heart Failure Management: Beyond the Medication' in the British Journal of Hospital Medicine 2017. (**Apendix S**) Section 1.15 was published as a review article entitled 'The interplay between heart failure, metabolism and body composition'[218] in the British Journal of Hospital Medicine 2016.(**Appendix S**)*

Chapter Two

GENERAL THESIS HYPOTHESIS AND RESEARCH OBJECTIVES

2.1 GENERAL HYPOTHESIS

CRT has been effectively demonstrated to improve cardiovascular outcomes for the majority of HFrEF patients. Biventricular pacing has been shown to modify complex molecular pathways that are associated with adverse cardiac remodelling seen in HFrEF, including the ECM and specific regulating miRNAs. A large problem for individual patients and healthcare providers is that CRT is a costly treatment and despite meeting the evidence based implant criteria a significant minority of patients do not respond. Many factors have been examined to see what best predicts of response for CRT. Unfortunately non-response rates remain unchanged and other variables remain inconsistently reported in the literature.

Circulating biomarkers of these complex molecular pathways can be dysregulated in HFrEF and can prognosticate for outcomes. We hypothesise specific biomarkers have the ability to predict patients' potential to respond to biventricular pacing. Cardiac ECM have dynamic properties and turnover alters HFrEF and circulating biomarkers of this system has been shown to prognosticate. Furthermore CRT has been shown to alter ECM turnover. Our hypothesis is that measuring individual or a combination of circulating ECM biomarkers before CRT implantation may predict a patient's functional respond.

Several miRNAs are involved in the regulation of complex cardiac molecular systems and are known to be dysregulated in HFrEF. We also hypothesise specific miRNAs expression may predict a patient's ability to respond to CRT. Together these circulating biomarkers may

have the strength to predict wehther a patient may respond to a CRT device before implantation.

CRT implantation in HFrEF patients offers a unique in-vivo model to examine maladapted complex cardiac molecular systems response to biventricular pacing and reverse cardiac remodelling. Limited information is available for ECM turnover and it is contradictory. MiRNA behaviour following CRT implantation is poorly understood and limited information is available. We hypothesise that CRT will alter ECM and miRNA biomarker profiles following implantation and the degree of alteration will depend on the patient's response status.

We also hypothesise that body composition is an important variable on predicting the response and outcome following CRT implantation. Determining the impact of CRT implantation on body composition is potentially important from a clinical perspective...

Finally, we hypothesise a prediction model will need to be individually tailored to the specific patient population. Specific clinical variables maybe more important than others in a HFrEF populations.

2.2 AIM

The aim of this research work is to undertake a proof-of-concept pilot project where traditional clinical variables are combined with novel circulating biomarkers to attempt to

predict HFrEF patients who are more likely to respond to CRT. The selection of ECM biomarkers and miRNAs is based upon the previous literature, including systematic review (**chapter 4**). These circulating biomarkers were tested in parallel to ellicit their value independently and as part of a wider prediction model. This study is similar to many others on CRT studies, which are based at a single centre and is hypothesis generating as the numbers of participants is low.

The design of the research focused in two areas to aid in building a real-world prediction model utilising relevant clinical variables and novel circulating biomarkers. The work was undertaken at the University Hospital Coventry and Warwickshire (UHCW) NHS Trust. The intricacies of the local HF population are discussed in **Chapter 3.2**. Firstly a retrospective study of all CRT implants was planned and undertaken as discussed in **Chapter 6.4**. The aims are summarised as follows and detailed in **Chapter 5.2**:

1. To examine pre-determined clinical predictors of CRT response, informed by the literature as to potential predictors within our local HFrEF population.
2. To inform clinical variable selection for inclusion in the prediction model alongside novel circulating biomarkers.

A prospective observational study was designed to test a prediction model using clinical variables and novel circulating biomarkers for CRT response. The principle CRT response definition is a functional one based on a combination definition, which is detailed in **Chapter 3**. The selection of novel circulating biomarkers to be tested for ECM is informed by the

systematic review detailed in **Chapter 4** and for miRNAs **Chapter 1**. The selected novel circulating biomarkers are outlined in detail in **Chapter 3**. The prospective observational study has been designed as a proof-of-concept pilot study to test the initial prediction model and offer an opportunity for refinement. Body composition was studied as a sub-study of the prospective observational study to understand the changes that occur following CRT implantation, which is discussed in detail in **chapter 7**. The aims of the prospective observational study are summarised below and discussed in **chapter 6 and 7**:

1. To characterise novel circulating biomarker expression in reduced ejection heart failure (with dyssynchrony) in cardiac specific and peripheral (systemic) baseline samples.
2. To characterise miRNA expression [cardiac specific and systemic] in reduced ejection fraction heart failure patients (with dyssynchrony) before CRT implantation and during follow-up
3. To characterise the ECM biomarker expression in reduced ejection fraction heart failure [with Dyssynchrony] before CRT implantation and during follow-up
4. To determine the impact of CRT implantation on body composition in patients with reduced ejection fraction heart failure (with dyssynchrony)

Chapter Three

GENERAL MATERIALS AND METHODOLOGY

3.1 INTRODUCTION

This chapter introduces and details all methodologies and materials utilised during this research project. Both main studies (retrospective and prospective) are discussed. A complete account of all protocols and techniques used are described in detail in this chapter. Specific methodologies and material used in each study are discussed with in the relevant section of this chapter and their results **Chapters 4, 5 and 6** respectively.

3.2 UHCW NHS TRUST CARDIAC RESYNCHRONISATION THERAPY IMPLANTATIONS

3.2.1 Local Service and Patients

This research has been conducted within the Arden Cardiac Network (**Figure 3.1**) with UHCW NHS Trust as the principle tertiary centre for CRT implantations in the network. UHCW directly serves more than half a million patients.[219] It also serves as the tertiary referral centre for George Elliot Hospital NHS Trust (Nuneaton) and expert centre for South Warwick Hospital NHS Trust, Worcestershire Acute Hospitals NHS Trust and County Hospital Hereford NHS Trust ,representing an estimated population of 1.6 million.[220] In recent years many centres have developed their own services.

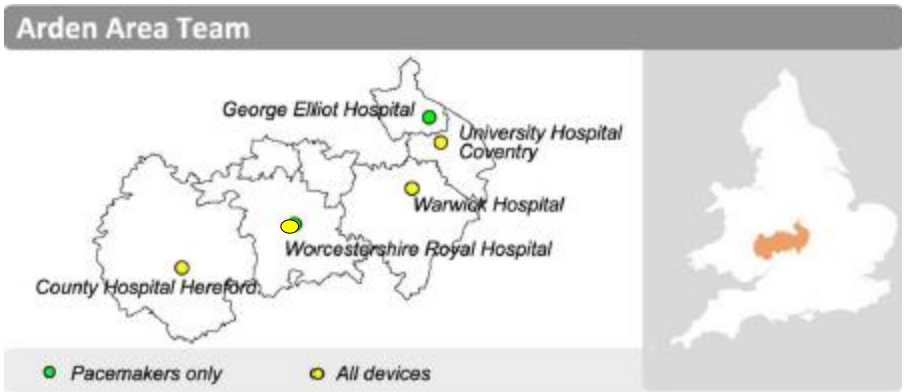


Figure 3.1 Arden Cardiac Network 2016. Taken from National Audit of Cardiac Rhythm Management Devices 2012 Report.[220]

The CHF population varies within the Arden geographical region, which is important in considering who the cohort in study II represents. The North Warwickshire region (including Coventry) has a 0.8% CHF prevalence, compared to 0.7% in England within 2014/15.[221] There are specific factors which differentiate the Coventry population with the rest of the Cardiac Network and nation. Firstly 25.9% of the Coventry population is Black, Asian or another minority group, which is higher than the regional and national proportion, which are 16.8% and 16.3% respectively.[221] The South East Asian population is known to have a higher proportion of cardiovascular disease in the UK.[222,223] The South Asian population accounts for 3.94% of the UK population, however it is 15.1% in Coventry and Warwickshire[219] Outside Coventry, the remaining Arden area has an older population, who proportionally meet requirements for CRT implantations, reflecting the need for higher implantation rates.[220] The dynamics of this single centres population needed to be accounted for in the prospective study design.

3.2.2 UHCW CRT Implant Service

CRT implantation rates at UHCW and within the wider Arden Cardiac Network have historically been lower than the national average (**Figure 3.2**), but have been increasing annually.[220] The increasing implantation rate in the network and nationally reflect the broadening of the national criteria, [17,47,56] increasing number of centres, improvement in technology and upskilling of more operators. Implantations of complex cardiac devices including CRTs started at UHCW in late 2008.[224] UHCW year-on-year has demonstrated in an increase in implants in the most recent National Audit of Cardiac Rhythm of Management 2013-14.[59] Our own data shows UHCW implanted 82 CRTs in 2010 and 86 in 2012,[224] a pattern which is reflected at the national level (**Figure 5.2**).

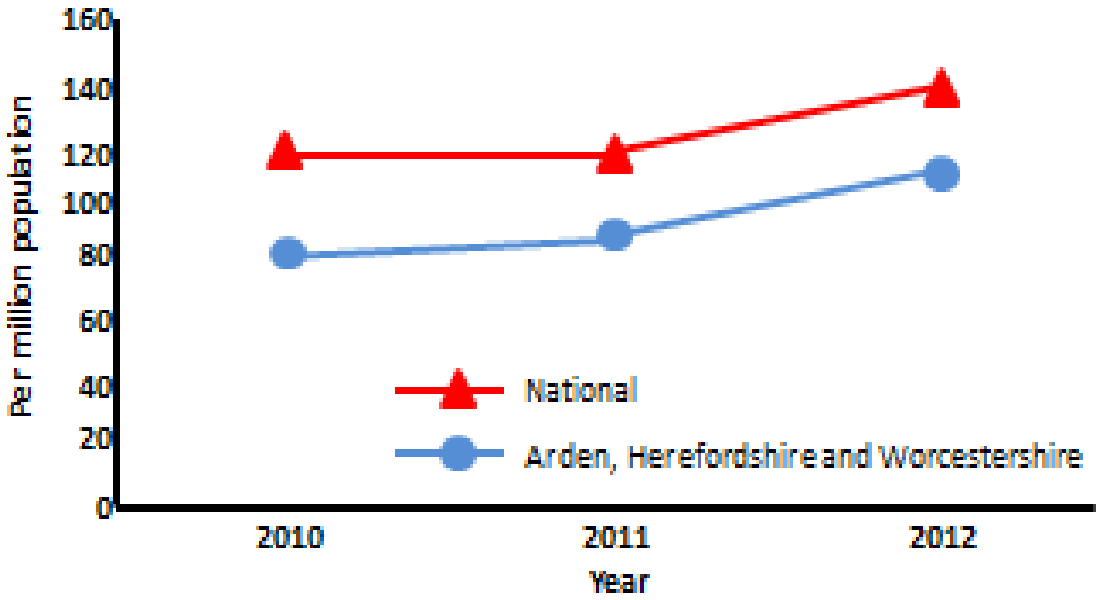


Figure 3.2 CRT implantation Rates in Arden Cardiac Network compared to National Rate 2010-2012. (Adapted[220])

The UHCW CRT service has evolved since 2008, with more elective implantations taking place. These elective implantations used to require planned overnight stays, but are now routinely performed as day-case procedures. We performed an analysis of our overall elective admission procedures complication and mortality rate (30 days and 1 year).[224] **Table 3.1** demonstrates our complication and mortality rates overall and by elective admission strategy. No significant difference between mortality and complication rates were identified between the different strategies.[224] Our analysis of elective complications demonstrated rates lower than the national average.[224,225] Complications however, would need to be accounted for in the prospective cohort study design as they would be likely to influence the results.

Elective patients from Coventry and Warwickshire are referred directly to the arrhythmia clinic for consideration for a CRT device. Emergent patients are assessed as inpatients for CRT. Often urgent patients can be discharged and have their procedures performed as an urgent outpatient. All patients require cardiac imaging, resting 12-lead ECG and clinical assessment for an implant decision to be made. Cardiac imaging is required to assess and quantify LVEF and LV dimensions; this was frequently achieved on transthoracic echocardiography. Patients were were assessed in the context of the the NICE guidance that was in place at the time (2007[51], 2010[226] or 2014[17]). **Table 1.2** demonstrates the current NICE 2014 guidelines.[17] The guidelines did not replace clinical decision making. Complicated cases were often discussed in the arrhythmia multi-disciplinary team meeting prior to an implantation decision being made.

Table 3.1 Elective Complex Cardiac Devices Complications (January 09– April 2013)[224]

Outcomes	Total Cohort (n=267)	Same-day (n = 213)	Overnight (n=54)	P Value
Failed Procedure	**7 (2.6%)**	**7 (3.3%)**	**0**	**0.4**
Unplanned overnight stay		**9 (4.3%)**		
Total Complications	**26 (9.7%)**	**20 (9.4%)**	**6 (11.1%)**	**0.8**
Immediate (≤24 hours)	9 (3.4%)	7 (3.3%)	2 (3.7%)	1.0
RV Lead Displacement (n)	2	1	1	
Diaphragmatic Stimulation (n)	2	2	0	
Pneumothorax (n)	1	1	0	
Haematoma (n)	3	2	1	
Wound bleeding (n)	1	1	0	
Short term (>24hrs-6 weeks)	6 (2.2%)	4 (1.9%)	2 (3.7%)	0.4
LV Lead displacement (n)	2	1	1	
RA Lead displacement (n)	1	0	1	
LV Lead not capturing (n)	1	1	0	
Wound infection (n)	1	1	0	
Pre-erosion (n)	1	1	0	
Medium term (6 wks-4 months)	4 (1.5%)	4 (1.9%)	0 (0.0%)	0.6
LV Lead Displacement (n)	2	2	0	
RV Lead Displacement (n)	1	1	0	
Device Infection (n)	1	1	0	
Long term (>4 months)	7 (2.6%)	5 (2.3%)	2 (3.7%)	0.6
LV Lead Displacement (n)	6	5	1	
LV Lead Not Capturing (n)	1	0	1	
Mortality:				
Mortality ≤ 30 days	3 (1.1%)	2 (0.9%)	1 (1.9%)	1.0
Mortality ≤1 years	24 (9.0%)	17 (8.0%)	7 (13.0%)	0.5

3.2.3 Implantation Procedure

All CRT implants were performed according to our centres standard operating procedure. The precise model of pulse generator and lead types implanted were determined by normal working practice and neither study affected their selection. Each procedure was performed or supervised directly by a Consultant Cardiologist with a specialist interest in Cardiac Devices. Most implants were left sided. The pulse generator was placed into a subcutaneous pre-pectoral pocket. CRT pacing/defibrillator leads were placed by an endocardial approach;

this was mostly via the cephalic or axillary veins. The subclavian vein was used if other routes proved unsuccessful. The RV pacing lead was placed at the RV apex (RVA) in the majority of patients and infrequently at the RV septum. The RA lead was implanted mostly at the RA appendage (RAA) and when this was not possible at the RA free wall. Patients in permanent AF did not have an atrial lead implanted unless an external cardioversion was planned in due course. The LV lead placement was via the CS. To identify the preferred deployment site the CS was cannulated and angiography performed to demonstrate the cardiac venous anatomy. The preferred LV lead deployment was the most lateral circumferential position and basal/mid-cavity axial position possible. Many patients with ischaemic cardiomyopathy have scar as a result of infarction. The scar can be identified by cardiac magnetic resonance imaging, LV lead placement is then avoided at this site as it can reduce the success of the procedure.[227] **Figure 3.3** demonstrates the preferred endocardial (RA and RV) and epicardial deployment and sites for a CRT device. Defibrillation safety margin testing was not performed on those having a defibrillator.

All were given pre and post-procedure intravenous antibiotics: Flucloxacillin 1g IV and Gentamicin 1.5mg/kg IV (max dose 100mg) followed by Flucloxacillin 500mg QDS orally for 3-days. If allergic to penicillin's, Teicoplanin 600mg IV pre-procedure and Doxycycline 200mg OD for 3 days. All implants had post implant chest x-ray and device check prior to discharge. The CRT implant protocol altered in 2010 for elective implants to day-case procedures being (admitted via the Day-Case Unit) and observed for 3-4 hours post procedure before being discharged, if all checks were satisfactory.[224]

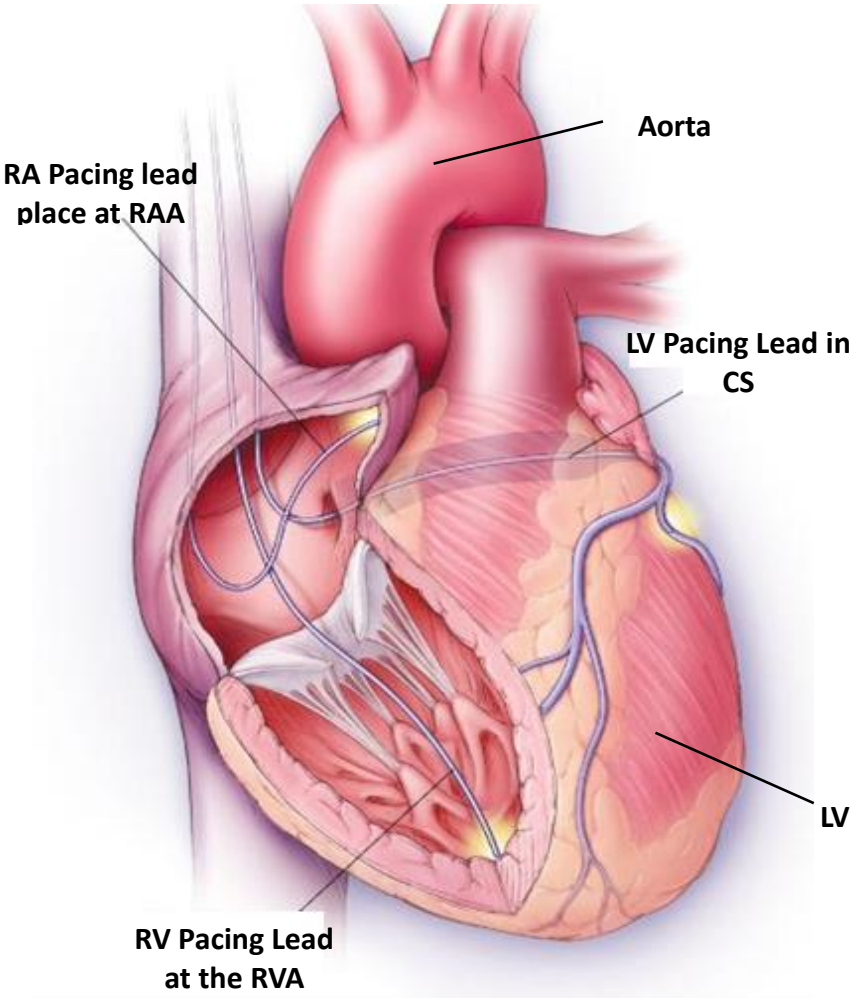


Figure 3.3 Cross Sectional View of Heart and Endocardial CRT Lead Placement. (Adapted[16])

3.2.4 Routine Aftercare

All CRT implant patients return for a standard six week clinical and device review. Patients are clinically assessed by the arrhythmia nurse team. Nurse led clinics are supervised by Consultant Cardiologists. The CRT device checks are conducted by cardiac physiologists for battery life, cardiac arrhythmias, lead impedance and thresholds. CRT-d devices are checked for any anti-tachycardia pacing and / or shock delivery. Patients referred from other centres were repatriated after the six week check. Long-term CRT devices are checked as a standard every 6 months.

3.3 APPLIED DEFINITIONS

3.3.1 Outline

Several definitions are applied throughout this project and are given in the section below for bundle branch block morphology and NYHA symptoms classification. Applied clinical definitions for ischaemic aetiology, cardiomyopathy, diabetes mellitus and chronic kidney disease are outline in detail in **Appendix C.**

3.3.2 Electrocardiogram and QRS duration

Resting 12-lead ECGs are performed routinely prior to assessment for CRT implantation. Prolonged/Broad was defined as a QRS duration >120msec (>3 small squares on ECG). Specific QRS duration was calculated on digital ECG's by measuring from the start of the Q wave and to the end of the S wave.[228] Manual digital measurements of QRS duration is now the gold standard technique to measure QRS duration.[228]

3.3.3 Bundle Branch Block

A broad QRS complex on a resting 12-lead ECG must meet specific morphological criteria to be defined as a complete LBBB or RBBB. **Table 3.2** describes the AHA/ACCF/HRS 2009 criteria for defining complete LBBB and RBBB.[229]

3.3.3.1 Nonspecific or Unspecified Intraventricular Conduction Disturbance[229]

NIVCD is defined as a QRS duration ≥110 msec without the criteria for LBBB or RBBB (**Table 3.2**) being fulfilled.[229] The definition can be applied to a pattern with RBBB criteria in the precordial leads and LBBB criteria in the limb leads, and vice versa.[229]

Table 3.2 Criteria for Complete LBBB and RBBB.(Adapted[229]*)*

Complete LBBB		Complete RBBB	
1	QRS duration ≥120 msec	**1**	QRS duration ≥120 msec
2	Broad notched or slurred R wave in leads I, aVL, V_5, and V_6 and an occasional RS pattern in V_5 and V_6	**2**	rsr', rsR', or rSR' in leads V_1 or V_2. The R' or r' deflection is usually wider than the initial R wave. In a minority of patients, a wide and often notched R wave pattern may be seen in lead V_1 and/or V_2
3	Absent q waves in leads I, V_5, and V_6, but in the lead aVL, a narrow q wave may be present	**3**	S wave > R wave OR > 40 msec in leads I and V_6
4	R wave peak time >60 msec in leads V_5 and V_6 but normal in leads V_1, V_2, and V_3, when small initial r waves can be discerned in the above leads	**4**	Normal R peak time in leads V_5 and V_6 but > 50 msec in lead V_1
5	ST and T waves usually opposite in direction to QRS	Of the above criteria, the first 3 should be present to make the diagnosis. When a pure dominant R wave with or without a notch is present in V_1, criterion 4 should be satisfied.	
6	Positive T wave in leads with upright QRS may be normal (positive concordance)		

3.3.4 Clinical Assessment

3.3.4.1 New York Heart Association Symptom Classification

The NHYA symptom classification is an ordinal scale used by clinicians to grade a patients HF symptoms. NYHA classification is consistently used in all CHF observational and randomised control trials to clinically assess symptom severity.[2] Furthermore, it is used consistently in all the CRT randomised control trials.[22,23] **Table 3.3** shows the NYHA symptom classification scores.

Table 3.3 NYHA Symptom Classification. Taken from McMurray *JJ et al*; Eur J Heart Fail 2012.[2]

Class I	No limitation of physical activity. Ordinary physical activity does not cause undue breathlessness, fatigue, or palpitations.
Class II	Slight limitation of physical activity. Comfortable at rest, but ordinary physical activity results in undue breathlessness, fatigue, or palpitations.
Class III	Marked limitation of physical activity. Comfortable at rest, but less than ordinary physical activity results in undue breathlessness, fatigue, or palpitations.
Class IV	Unable to carry on any physical activity without discomfort. Symptoms at rest can be present. If any physical activity is undertaken, discomfort is increased.

The NYHA classification is often used as a metric of severity of illness and assessing response to treatments for CHF.[2] There is a well described relationship between severity of symptoms and survival, however even patients with mild symptoms still have a high absolute hospitalisation and mortality rate.[230,231] NYHA classification is known to poorly correlate with ventricular function.[2] Application is often inconsistent; Raphael *et al*[232], performed an inter-observer variability study and reported a 54% concordance for two Cardiologists in grading 50 patients. Progression through the ordinal NYHA scale varies on whether acute or chronic illness is present at the time; acute illness often demonstrated a repaid decline and with treatment, a rapid improvement.[2]

Throughout this study NYHA symptom classification is an important metric for assessing and grading symptoms at a particular time-point. It forms an important outcome variable in both the cohort studies.

3.4 STUDY I: RETROSPECTIVE COHORT STUDY

5.4.1 Title

Evaluation of Potential Pre-Implant Predictors of Clinical Response and Cardiovascular Outcome for Cardiac Resynchronisation Therapy

3.4.2 Indication for Study

An examination of the historic implantation procedure at UHCW was important for informing decisions about analysis of the prospective study design and analysis within the context of the study population. Examining the local population and more importantly the previous CRT implantations informed the prospective study ahead of recruitment of the typical patients that had CRTs implanted at our study centre. Studying the previous implants also informed the study feasibility.

3.4.3 Rationale for the Retrospective Study

UHCW was the site for the prospective study (Study II) and it offered particular population characteristics that had to be accounted for within the subsequent study analysis. Performing a study on the historical implants offered an opportunity to understand the specific CRT implant population within Coventry, Warwickshire and the wider Arden Cardiac Network. The differences in the general population are important in order to consider how they might influence determination of CRT response and cardiovascular outcomes. The

earlier analysis determines that the population is characteristically different to the regional and national population due to multiple factors. Moreover our own procedural analysis determines that our experience in terms of outcomes from our procedures is not only comparable at a national level but is evolving as new evidence and guidelines become more apparent and techniques improve. Studying previous CRT implantation patients at UHCW informed the prospective study as it is the closest analysis that reflects the cohort samples that will be achieved for the prospective cohort. Specifically pre-implant predictors we might want to factor into our prospective study prediction models were determined. Moreover, the stability in outcomes for CRT implantations, demonstrates the stability of implants at UHCW and the potential number of patients that might be expected to drop out due to procedure failure or an early complication.

3.4.4 Study Governance

An application was made to the Trust's Research, Development and Innovation department for ethical review under section 2.3 of the harmonised UK-wide edition of the *Governance Arrangements for Research Ethics Committees*[233] for permission to conduct and perform our proposed retrospective study. Permission was granted to review patient records and analyse anonymous patient data. Approval was provided by our local research, development and innovation department. The study applied the principles of the declaration of Helsinki.

3.4.5 Study Overview

3.4.5.1 Design

This is a single-centre, unselected retrospective cohort study of all consecutive CRT implants at UHCW performed over five years (January 2009 to December 2013). Patients were observed until 31st December 2014. **Figure 3.4** summarises the study schema.

3.4.5.2 Patient Screening

All CRT implantations were screened. Patients had to meet the national and international implantation guidelines active at time of the procedure to be included in this anonymous retrospective cohort study.[17,47,226,234] All CRT-p and CRT-d implants both *de novo* and upgrade implants were included. **Figure35.4** outlines the screening and specific exclusion criteria. Immediate complications were excluded as the patient was required to have instanous biventricular pacing achieved as not to bias assessment of response.

3.4.5.3 Patient Selection

Electronic / paper case records for Cardiology / Heart Failure consultations had to be available and contain the information to conclude a NYHA classification for that patient at that contact time. **Figure 5.4** summarises the process of patient selection.

3.4.5.4 Patient Consent

For the retrospective study under *Governance Arrangements for Research Ethics Committees*[233] patient consent was not required, as only anonymous data is recorded.

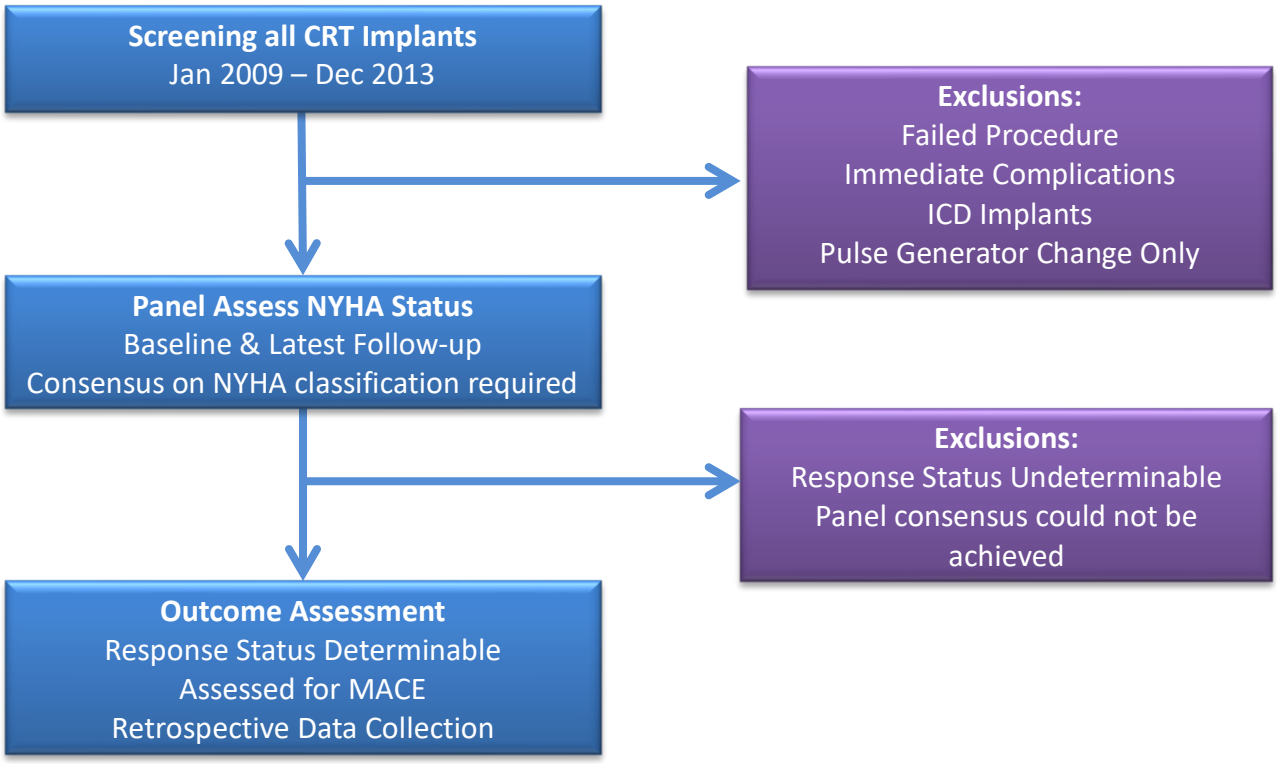


Figure 3.4 Patient Screening, Selection and Study Process

3.4.5.5 Investigations

For the retrospective study no patient investigations were possible and all information was be obtained from sources that have already recorded information.

3.4.6 Clinical Data Acquisition

3.4.6.1 Data Sources

Electronic (Clinical Results Reporting Systems., University Hospital Coventry and Warwickshire, Coventry, UK)/Paper case note records and hospital coding data were utilised to search for demographic, medical background, ECG, transthoracic echocardiogram reports, procedure information and outcomes. Referrals from external centres in the Arden Cardiac Network (**Figure 3.1**) were included in the analysis and information in referral letters

were utilised to complete data collection for those patients. Procedure reporting systems (Carddas, GE Healthcare, Horten, Norway) were also searched for information. Echocardiogram digital storage software (EchoPac, GE Healthcare, Horten, Norway)) was searched for echocardiogram reports and available images.

3.4.6.2 NYHA Pre-Procedure and Follow-up classification

Availability of NYHA symptom classification score (**Table 3.3**) pre-procedure and the latest review by a cardiologist/heart failure team member was reviewed initially as part of the screening and selection process. The 'latest' consultation was defined as that closest to the final observation date (31st December 2014). Patients with clearly labelled NYHA symptom class at both time-points were included and had available data recorded. Those with a description of symptoms, but no clear NYHA classification were taken to the 'NYHA Consensus Panel' (discussed later in this section).

3.4.6.3 Data Collected

The defined data collection period was from the date of the CRT implantation procedure until the 31st December 2014 or until mortality or a loss to follow-up. Two investigators separately reviewed all records for each patient included in the study. Specific data variables collected are outlined in **Appendix D**.

3.4.6.4 Electrocardiograms

All CRT implantations patient underwent a resting 12-lead ECG to determine rhythm, QRS duration and BBB morphology to see if the criteria for implantation had been met. [17,51,226]

All digital / paper ECG's available were sought for those patients in the study. The ECG had to be performed before the implant. ECGs were reassessed for QRS duration[228] and BBB morphology.[229] QRS duration was performed on the digital records where available. These measurements were performed by the lead investigator. This process was designed to minimise the variability in measurements.

3.4.6.5 Transthoracic Echocardiogram

All transthoracic echocardiogram images for patients included in the study if available were recovered from the digital archive. The original reports were not reviewed. Measurements were performed on post processing software (EchoPac, GE Healthcare, Horten, Norway). Specific measurement of LV end systolic and end diastolic volumes (LVESV and LVEDV) were performed in the apical four and two-chamber views. These measurements were used to calculate the LVEF via the modified Simpson's Biplane method. Standard methods[201] were used to perform all the required measurements. Recorded images had to be good enough quality to perform these measurements to be used. The lead investigator performed all of the measurements. If any new findings were identified on evaluation the lead author would inform the clinical consultant for the patient and allow them to directly action if needed.

3.4.7 Potential Pre-implant Predictors Model

Potential clinical predictors were considered prior to data collection and pre-selected based on previous reports (**Chapter 1.3**). Predictors identified were age and gender,[38] device type

(CRTp/CRTd) and upgrade status,[235] clinical aetiology,[38,65,68] CKD,[236-238] diabetes mellitus,[236] BBB morphology,[38,66,68] QRS duration,[38,39,68] and LV ejection fraction on echocardiography.[38,67]

CKD status at implant was defined as an eGFR (estimated glomerular filtration rate) <60ml/min/1.73m^2 using the modification of diet in renal disease equation.[237,239,240] Baseline renal function was recorded, but was considered to have potentially high levels of missing variables, hence hospital coding data was utilised to define CKD status. Time from implant to determination of response was considered likely to be wide, therefore, time between implant and assessment was included as a confounding variable in the clinical predictor model.

Pre-procedure NYHA symptom classification status was not included as a predictor due to the direct association with clinical response. Implantation factors were not included as they were not determinable prior to implant and would have no value in predicting response pre-implant.

3.4.8 Outcomes

3.4.8.1 Overall Clinical Response

The primary outcome was the overall clinical response assessed at the latest cardiology/HF consultation. The difference in NYHA symptom classification score between pre-procedure and at the latest consultation were used to determine clinical response. The criterion for clinical response was a decrease in NYHA classification $\geq$1 symptoms from baseline.

Determination of NYHA symptom classification was performed by reviewing all recorded consultations. A secondary analysis examined clinical response status determined acutely (≤12weeks) and long-term (>12weeks) based upon the latest cardiology/HF team review in the defined time periods. Overall clinical response was used as the primary outcome and utilised the latest cardiology/HF review for the patients in the observation period, with information available to define NYHA symptom classification score.

3.4.8.2 Clinical Response Assessment Panel

Separate reviews of all electronic/paper case notes was performed by three clinicians (based at UHCW) experienced in assessing CHF patients. The reviewers were blinded to other reviewers. A consensus was required for each patient based upon reported evidence at baseline referral for CRT implantation and latest follow-up within the observation period. Only correspondence or documentation from a Cardiologist or HF team member was used as evidence of assessment. Outpatient clinics were the preferred clinical engagement to assess. Often clinicians recorded a specific NYHA symptom classification at the time of the assessment, in these cases this numeric value was used. When a specific case was not classified, the reported symptoms were studied. Independent clinical assessment of evidence was performed to judge an NYHA classification score. A description of symptoms and exercise tolerance was required to be documented to reach a judgement. A high evidence threshold was used to minimise the bias of this process. The consensus was also required between the reviewers given the recognised high variability in opinion in assessing NYHA classification.[232]

3.4.8.3 Major Adverse Cardiovascular Events

MACE was a secondary outcome for this project. MACE is a clinical composite of all-cause mortality and/or first HF hospitalisation.[39] This definition is applicable for both aspects of this project. HF hospital admissions were defined as a hospital admission requiring intravenous diuretics. All-cause mortality rates were generated from hospital coding data and electronic patient records.

3.5 STUDY II: EXPLORATIVE PROSPECTIVE STUDY

2.5.1 Title

The Characterisation of Circulating biomarkers before and after Cardiac Resynchronisation Therapy in patients with Chronic Heart Failure and their Role in Predicting Response (The COVERT-HF Study)

3.5.2 Research Governance

3.5.2.1 Research Study Ethical Approval

The application to the West Midlands REC was submitted using the online Integrated Research Application Service portal (IRAS application number: 135985). The South Birmingham REC meeting was attended by Dr Faizel Osman and I on the 30th September 2013 where the proposed study was discussed. The REC consisted of a multi-disciplinary panel of clinicians, academics, healthcare professionals, statisticians and lay members. Following minor amendments to the protocol, permission was granted to perform the study on the 30th October 2013 (**Appendix E**). Subsequent permission was granted from the UHCW Research, Development & Innovation department to conduct the study. (REC

Number: 13/WM/0355). Subsequent amendments to the ethical approval are discussed in detail in **Appendix E.**

3.5.2.2 Clinical Trial Registration

The COVERT-HF prospective study was registered on the publically accessible Clinical Trials (www.clinicaltrials.gov) database (Registration Number: NCT02541773). **Appendix F** shows the uploaded Clinical Trials database information.

3.5.2.3 Patient and Public Involvement

Patients have been actively involved and were essential to the conduct of the research project. During the planning of the study and application to the South Birmingham Research Ethics Commission, patient involvement has been central. Five patients, who were listed for CRT implantation, were questioned to determine their opinion about the study. An assessment of how the study design reflected their health beliefs and whether uptake rate would be influenced by the study design was evaluated during these interviews.

A subsequent consultation took place with the local comprehensive research network patient group in March 2014 to discuss the study and its methodology. This built on the initial patient involvement and comments were passed on the study design and the material being used, to recruit and collect data.

The importance of reporting these findings to the specific participants and to HF patients is important. We have sent a summary document of the research findings to each surviving

research study participant and have presented them at the Rugby Cardiac Rehabilitation group [www.rugbytakeheart.org.uk].

3.5.3 Study Participants

3.5.3.1 Participant Screening

All patients undergoing CRT implantation at UHCW were screened against the eligibility criteria (section below). If the criteria were met the patients were approached to participate ahead of implantation. The COVERT-HF study did not alter or affect the decision to offer a CRT or the type of device. Screening occurred once the clinical decision had been made. Evaluation of the previous implants at UHCW suggested that recruiting 50 patients in two years was both feasible and achievable for this Proof-of-concept study.

3.5.4 Study Outcomes

The criteria for CRT functional and echocardiographic response will be applied short-term [six week review] and long-term (six months). Determining participant functional and echocardiographic status only occurred following the final follow-up study visit for the entire cohort and data-lock after database quality check .

3.5.4.1 Response Definition

Table 3.4 demonstrates the functional and echocardiographic response criteria's that were utilised in the prospective observational study. The functional response criteria formed the

primary outcome. An echocardiographic definition was used as a secondary definition. The definition used for response did not combine functional and echocardiographic criteria's as it is well established these variables poorly correlate.[1] composite definition is required for a small obersational cohort study.

The functional response definition used a composite definition. The functional response definition reflected symptoms, function and QoL.[1] Three specifc criteria formed the combination definition. At least two of three parameters had to be present to determine a functional response. Symptoms were assessed using the NYHA classification, which is the commonest measure in the literature.[1,23,29,76,85,86] QoL was measured using the validated tool; the Minnesota Living with Heart Failure Questionnaire (MLHFQ) to detect significant changes. Function was assessed using the change in 6MWT.distance.[241] LV remodelling has been defined by a echocardiographic variety of LV geometric and function measurments.[1] The most widely used echocardiographic measure of response is a ≥15% reduction in LVESV.[52,71,242-244] This specific measure has been used to define echocardiographic response.

All definitions are based upon the difference between baseline and 6 months follow-up (follow-up measurement - Baseline measurement). Both clinical and echocardiographic criteria had HF end-points (mortality or heart transplant) built in to define non-response, so those participants were not lost to follow-up. Including absolute end-points in definitions is common in more recent observational studies[1,65,66,68] and minimises missing data.[245]

Table 3.4 Prospective Clinical and Echocardiographic Response Criteria

Clinical Response at 6 months
Two out of Three: ↓≥ 1 NYHA ↓MLHFQ score > 5 ↑≥ 10% 6MWT distance
Echocardiographic Response at 6 months
↓≥ 15% LVESV

3.5.4.2 Major Adverse Cardiovascular Event

The same MACE definition has been applied throughout the entire project. A definition of MACE is given earlier in this chapter. In COVERT-HF participants were observed for MACE for 12 months. Participants at each follow-up study visit (**Figure 5.5**) were asked about MACE. Approaching twelve months electronic/paper case notes alongside hospital coding data was reviewed. Participants were also contacted to ask about MACE.

3.5.5 Eligibility Criteria

Participants must have clinical investigations to allow assessment under the clinical criteria of the NICE guidance for CRT implantation.[17] LVEF can be originally evaluated by echocardiography, cardiac magnetic resonance imaging or nuclear myocardial perfusion scanning (this does not replace baseline echocardiographic evaluation).

3.5.5.1 Inclusion Criteria

1. Age > 18 years
2. LVEF ≤35% on echocardiography
3. NYHA Class III/IV symptoms or milder symptoms with:
    a) NYHA I (LVEF ≤35% and QRS≥150msec on resting electrocardiogram)
    b) NYHA II (LVEF ≤35% with either QRS≥150msec or QRS 120-149msec with LBBB on resting electrocardiogram)
4. Optimal medical therapy for heart failure that the patient tolerates (ACEi, Beta-Blocker, Mineralocorticoid) for > 3 months
5. QRS duration ≥120-149msec with LBBB on resting ECG **or** QRS duration >150msec on resting ECG
6. Patient consent to participation in the study

3.5.5.2 Exclusion Criteria

1. Acute heart failure decompensation ≤ 6/52 before implant
2. Significant cognitive impairment
3. Acute coronary syndrome ≤ 6/52 before implant
4. Chronic kidney disease stage V (requiring dialysis)
5. Terminal illness with likely survival ≤ 1 year after implant

3.5.5.3 Procedure and Post Procedure Study Exclusions

1. Failure of procedure (e.g. CS anatomy)

2. Complication resulting in poor/ none biventricular pacing (e.g. phrenic nerve stimulation, lead displacement/ damage)

3.5.5.4 Informed Consent

Patients were approached once decisions had been made regarding CRT implantation had been made. Patients were always approached in person by the investigator with ample time before the prospective implantation being performed. A patient information leaflet (**Appendix G**) was provided at this discussion, which outlined the study. Each patient was given adequate time to consider participation in the study and ask any questions they might have. Only once a patient was completely satisfied, was the patient asked to complete a research study consent form (**Appendix H**). Copies were provided for the participant, medical notes, General Practitioner and research notes. Enrolment in the study started in November 2013.

3.5.6 Study Design

3.5.6.1 Overview

COVERT-HF was an unselected, prospective cohort study of CHF patients referred for CRT placement at UHCW. The COVERT-HF study as outlined in the previous study was a proof-of concept study examining the potential predictive strength of specific ECM and miRNA biomarkers for CRT response. The research protocol is provided in **Appendix I**. All patients undergoing CRT implantation were screened, as previously outlined. The study was

performed over 32-months between November 2013 and June 2016. Recruitment occurred over the first 20 months with the final 12 months used to complete follow-up assessments and observe for MACE outcomes. A target to recruit 50 patients was set on the basis of the UHCW implantation rate (82 CRT implanted 2012).

Figure 3.5 outlines the COVERT-HF study schedule. Following recruitment to the study participants underwent three assessments at different time points (pre-procedure, 6 weeks and 6 months post-procedure). The pre-procedure assessment could be performed up to seven days before the procedure, but was planned to be done on the day of implant. The follow-up assessments were scheduled to take place simultaneously with routine CRT device reviews. Flexibility around the time of follow-up was allowed to be pragmatic and ensure participant retention. The specific ECM and miRNA biomarkers being investigated are stated **in Figure 3.5**. The evidence for their selection is outlined in **Chapter 1 and 4** respectively.

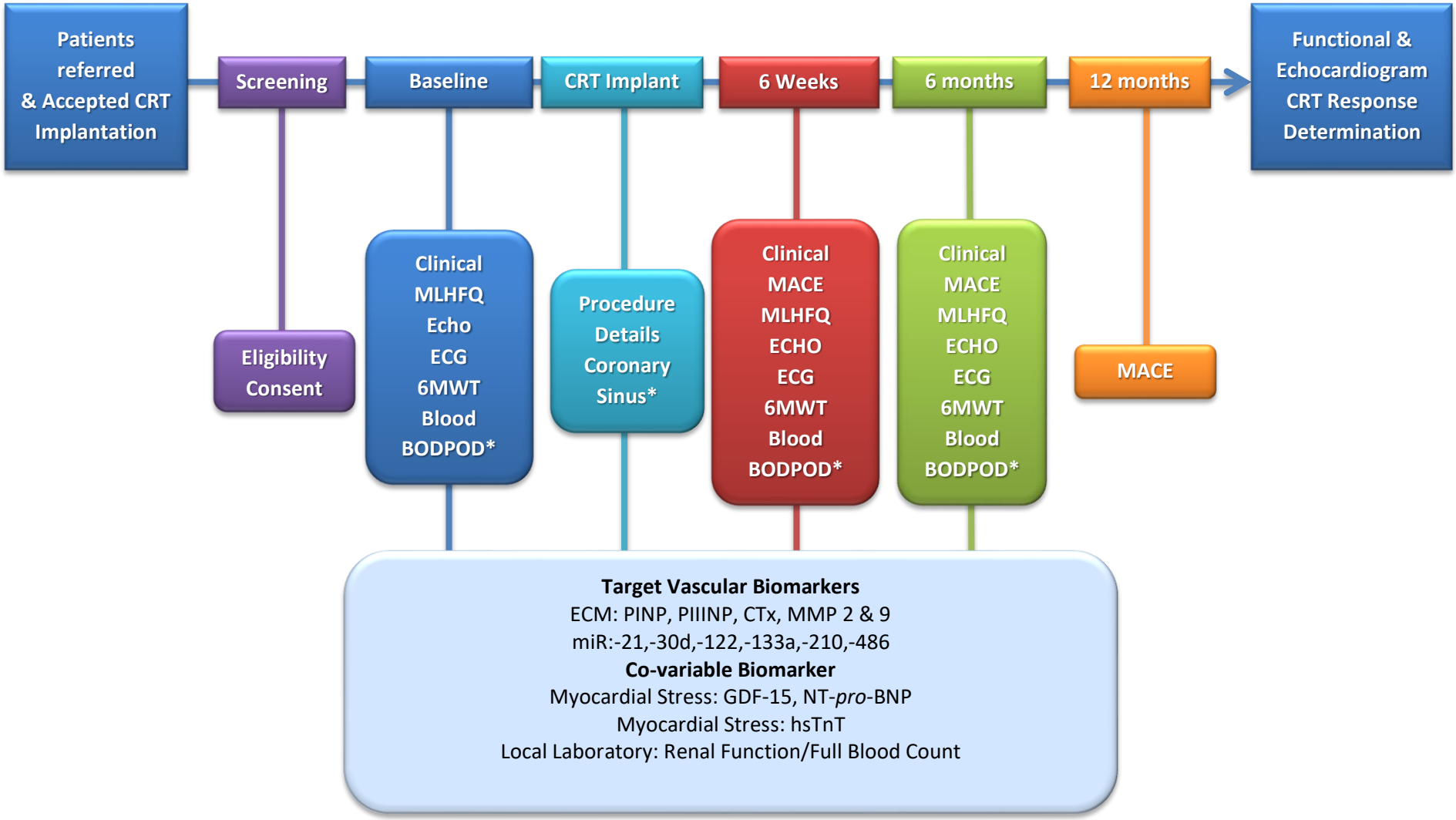


Figure 3.5 Study Scheme for COVERT-HF. *=performed on the second half of cohort

3.5.7 Assessments

Specific assessments were performed as per the study schedule (**Figure 5.5**). Clinical care for CHF and CRT continued with no changes to usual care.

3.5.7.1. Clinical Assessment

Appendix J outlines the specific clinical data collected at each study visit outlined in **Figure 3.5**. There was slight variation in data collected between the baseline and follow-up visits. Specific procedure details were also collected. All the data capture forms for baseline and follow-up study visits are shown in **Appendix K**.

3.5.7.2 CRT Device Interrogation

Routinely at 6 weeks and 6 months following the procedure, the implanted CRT was interrogated to ensure the device optimisation and to identify any complications. The interrogation of the implanted CRT is non-invasive using a programmer for the specific pulse generator in-situ (2090, Medtronic, Minneapolis, Minnesota, USA or Merlin 3650, St Jude, St Paul, Minnesota, USA or Zoom Latitude, Boston Scientific Natick, Massachusetts, USA). The interrogation of the device was performed by an accredited cardiac physiologist as part of the patient's routine care.

The biventricular pacing percentage and battery longevity was recorded. Any reprogramming that was performed was documented. Device complications that were identified were noted. Those patient with a CRT-d implanted also had defibrillator

functioning documented, specifically any anti-tachycardia pacing and/or shocks that had been delivered. This information alongside the clinical observations allowed the post-procedure exclusion criteria (**Section 5.2**) to be examined and applied if necessary.

3.5.7.3 Cardiovascular Observations

During each clinical assessment, a pulse rate was taken manually by palpating the radial pulse for one minute. A blood pressure reading was taken after 10 minutes of resting in a chair, obtaining a reading using a validated oscillometric sphygmomanometer (V100, Dinamap technology ®, GE Healthcare, Milwaukee, Wisconsin, USA). The same technique was performed for assessing blood pressure before and after the performance of the 6MWT.

3.5.7.4 Body Composition

Every participant underwent height and total body weight measurement using a standard combined height/weight scale (SECA, model 701/7021094, Germany). These measurements allowed calculation of BMI (weight (kg) / height(cm^2)). These measurements are in addition to the Air-displacement plethysmography measurements performed.

3.5.7.5 Electrocardiogram

All participants had a resting 12-lead ECG recorded using an electrocardiograph (MAC* 5500 HD Resting ECG System, GE Healthcare, Horten, Norway) as per the study schedule (**Figure 5.5**). Lead placement was according to standard practice and performed with all subjects in

the supine position by an experienced cardiac technician. Digital storage of the ECGs was performed for all traces. Interpretation of the ECG was by the lead investigator. Data was recorded (**Appendix K**) on rate, rhythm, pacemaker presence, PR interval, cardiac axis (normal/abnormal) and QRS duration/morphology on the clinical data capture form for both baseline and follow-up study visits. Interpretation of QRS duration and morphology applied previously described definitions in the literature.[228,229]

3.5.7.6 Six Minute Walk Test

The 6MWT is a simple and robust functional exercise capacity assessment that is easily performed, well tolerated by patients with cardiovascular and respiratory health problems, and reflects the patient's activities better than other walk tests.[241,246] It measures the functional exercise capacity globally and the involvement of multiple body systems, including the cardiac, pulmonary, circulation, blood transport and neuromuscular systems. In contrast to more complex functional exercise capacity testing (ie Cardiopulmonary exercise testing) specific measurements of these body systems cannot be performed.[241] Despite these obvious limitations, the 6MWT distance does correlate with peak oxygen uptake (r=0.73) in end-stage lung disease.[247] Walk tests reflect self-limiting sub-maximal exercise testing and reflect a patient's activity of daily living.[247] The 6MWT has specifically been shown to be a robust sub-maximal functional exercise test in patients with cardiovascular and respiratory impairment.[241,246,248,249] The 6MWT is well established for functional exercise capacity assessment in CHF[248] and has been used in the majority of randomised control trials examining CRT intervention.[23] In CHF patients undergoing CRT, the 6MWT has been demonstrated to be safe. [22,28,250] The 6MWT is a robust test in examining

changes in functional exercise capacity following an intervention.[241] Validity of the 6MWT has been shown to be high with short-term distance replication being excellent when circumstances are the same, including the investigator performing all the tests.[241,251]

All 6MWT were performed in concordance with the *American Thoracic Society* 2002 guidelines.[241] **Appendix L** provides a summary of the specific protocol undertaken performing the 6MWT and the information collected for the COVERT-HF study. 6MWT were performed at all patient assessments (**figure 3.5**). Two investigators undertook each 6MWT assessment with each patient, one of who was always a physician with an *Advanced Life Support* qualification. **Figure 3.6** demonstrates an example of a 6MWT being performed. The investigators were trained to perform the test by technicians who were experienced. The same investigator led the 6MWT for every test that was performed during the COVERT-HF study. Practice tests were not performed due the limitation of patient time on baseline assessment and the limited improvement in technique and 6MWT distance recorded on previous studies.[241,252]

Figure 3.6 A Replicated Example of a 6MWT.

The *American Thoracic Society* 2002 guideline[241] lists absolute contraindications to 6MWT as unstable angina or MI during the previous month. Acute heart failure hospitalisations in the last month were also treated as absolute contraindications. Relative contraindications are listed as resting heart rate of more than 120bpm, a systolic blood pressure of more than 180 mmHg, and a diastolic blood pressure of more than 100 mmHg. The physician supervising the test or the participant had the power to decide not perform or to stop the 6MWT.

3.5.7.7 Transthoracic Echocardiogram

A full transthoracic echocardiogram (Vivid 7, GE Healthcare, Horten, Norway) was performed at each study visit by a *British Society of Echocardiography* accredited

sonographer meeting the national standard.[253] All echocardiograms were performed in the Department of Cardiac Investigation at UHCW on the same machine by the same operator. Two dimensional echocardiography, myocardial tissue Doppler velocities and Doppler blood flow measurements were performed on each participant (**Figure 3.7**). Immediate review of all images was performed by the operator to ensure no significant clinical findings needed to be actioned. All measurements were analysed offline (EchoPac, GE Healthcare, Horten, Norway) by an unblinded investigator following a significant period of time after the scan (>1month). To partially mitigate for this reporting bias an inter-rater study was undertaken**.**

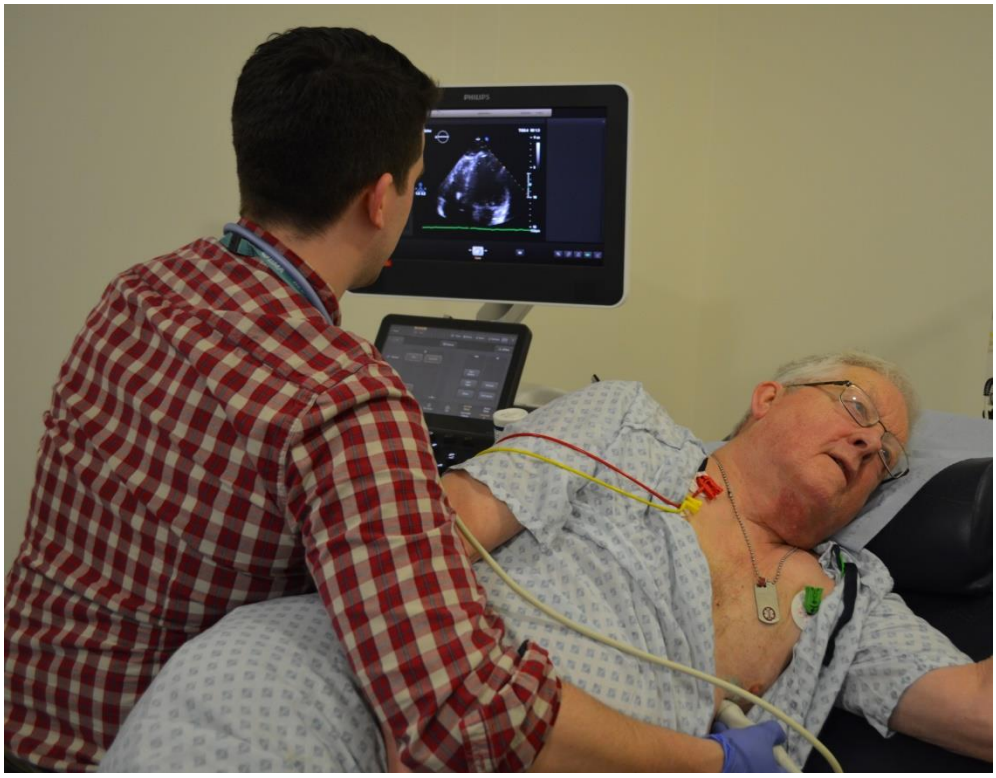


Figure 3.7 A Transthoracic Echocardiogram on a Participant. Participant consent obtained for use of image in research and publications.

A full set of measurements were performed in each study. Emphasis was placed upon obtaining two dimensional LV systolic and diastolic volumes in the four and two-chamber views using the biplane method of discs (modified Simpson's method).[253] The biplane method of discs is used to calculate the respective volume through the summation of a stack of elliptical discs that are produced when tracing the endocardial border during measurement analysis.[253] **Figure 3.8** demonstrates the images required to be taken to perform LVEF calculation using modified Simpson's method. LVEF was calculated using the biplane LV volumes[253] (LVEF = (LVEDV-LVESV)/LVEDV). Single plane measurements pose particular limitations when utilising this method to calculate the LV volumes when regional wall motion abnormalities are present.[253] The significance of LV dimensions and functional measurements is shown in **appendix M.**

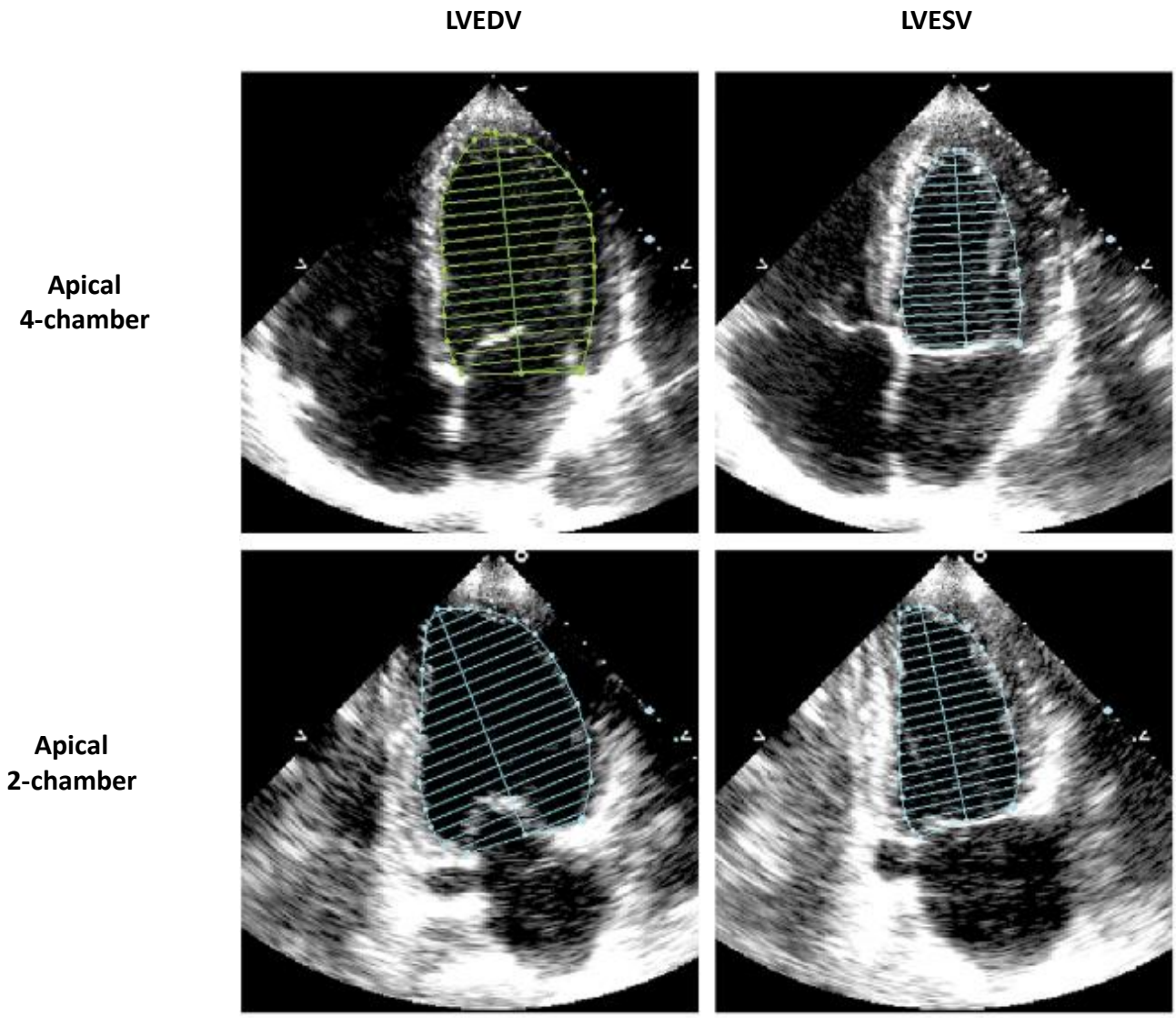


Figure 3.8. Two Dimensional Biplane Method for LV Measurement. Apical four- and apical two-chamber measuring LVEDV and LVESV respectively. Measurements biplane method of discs (modified Simpson's rule), and allow calculation of LVEF. (Adapted[253])

Diastolic function was assessed utilising two-dimensional measures, colour flow Doppler's and pulse and continuous wave Doppler Left atrial volume was calculated from the apical four chamber view.[254] Mitral value inflow measurements were performed in the apical four-chamber views and placing pulse wave Doppler at the coaptation point. During diastolic

filling of the LV, peak passive filling I and peak active filling (A) were measured and allowed the calculation of the E/A ratio. LV compliance was measured by Tissue Doppler imaging of the mitral annulus. [254] Pulse wave Doppler sampling is placed at or within 1cm of the lateral and septal annular sites during Tissue Doppler Imaging in the apical four-chamber view.[254] The peak passive filling velocity (e') and peak active filling velocity (a') were recorded. An average of the e' and a' were taken from the lateral and septal to calculate the averages.[254] The ratio of early transmitral flow velocity to the mean annular mitral tissue velocity (E/e') was calculated and represented an estimate of the LV end diastolic pressure.[254] Participants in AF at the time of the scan had an average for all measurements over 3-5 cardiac cycles. Measurements were not taken on an ectopic of the following heartbeat.

3.5.7.7.1 Inter-Rater Echocardiogram Variability Study

Echocardiography performance and reporting were conducted by an unblinded investigator. Despite the temporal dissociation for reporting scans built into the study design, there was a reporting bias present. Blinding for performance and reporting of echocardiograms was not feasible within the pragmatic limitations of the study designs. An inter-rater variability study was undertaken examine for the degree of homogeneity between two observers.

Twenty percent of echocardiograms were randomly selected to have LV volumetric (Biplane LVESV and LVEDV) and LVEF measurements repeated by a second independent and accredited sonographer (Mrs Kamerjit Rai). Block randomisation of every sequential five participants in order of recruitment was performed to select one to be included in the inter-observer study. All the echocardiograms that participants had performed were included in

the study. Methods used to assess inter-observer variation have previously been described in detail.[255,256]

3.5.7.8 Questionnaire

Participants were asked to complete the study questionnaire at all three study visits (**Figure 3.5**). Each questionnaire asked about participants symptoms and exercise tolerance. The baseline visit questionnaire additionally inquired about demographics gender, age and ethnicity. The two follow-up study visit questionnaires both enquired about any hospitalisations and device complications. Specific details of either of these events were explored. **Appendix N** demonstrates the symptoms questionnaire.

The MLHFQ was performed at all defined three study visits to assess participants QoL. The MLHFQ is a validated and reliable QoL questionnaire in HF research studies. The self-completion MLHFQ was administered to the participant as described in the literature.[257-259] **Appendix O** summaries how the questionnaire works and is scored. **Appendix P** shows the MLHFQ used in the study.

The MLHFQ was administered at the start of the research visit. An explanation given to the participant explaining how to answer the questions and that all questions must be completed was given at each research visit. When questions were not applicable it was advised to give a score of 0 as per the guidance.[258,259] The investigator left the room while

the MLHFQ was completed, but was available for clarification if needed. It was made clear to the participant that no aid in answering questions could be provided. A visual inspection was made of the score sheet upon completion to ensure no answers had been missed. Scoring was performed following completion of the research visit. **Appendix O** summarises the different domains and how the MLHFQ is scored.

3.5.7.9 Air-displacement plethysmography (Sub-Study)

Initially during the design of the COVERT-HF, body composition assessment was going to be assessed at each study visit (**Figure 3.5**) using the bioelectrical impedance analysis (BIA) method. BIA was considered due to availability to an Inner Scan®V, (Model BC-601, Tanita Corporation, Tokyo, Japan). CRT manufacturers St. Jude Medical (St.Paul, Minnesota, USA), Medtronic (Minneapolis, Minnesota, USA), Boston Scientific (Natick, Massachusetts, USA) advise that use of BIA is not recommended due to concerns over the possibility of oversensing, inappropriate shocks, inhibition of pacing, or device malfunction.[260] However, there is no evidence of interference from BIA devices on ICD function including telemetry disruption or lead oversensing.[260] Despite this evidence the manufacturer recommendation meant we were unable to perform body composition assessment with BIA.

COVERT-HF recruitment had begun when the decision to not perform BIA was taken. Whole body air-displacement plethysmography (BOD POD®) was considered to be a practical alternative to the more traditional techniques of measuring body composition and it would

be safe to perform for patients with CRTs *in situ*. The Human Metabolic Research Unit (HMRU) is a unique research facility designed to study human metabolism at our institution. Body Composition assessment is one of the facilities in the HMRU. The BOD POD® (Life Measurement Inc, Concord, California, USA) within the HMRU was used to perform the body composition assessment. **Figure 3.9** shows the Bod Pod in the HRMU at UHCW. **Appendix Q** summarises why the BOD-POD® is considered to be a very reliable and reproducible measurement of body composition.

Figure 3.9 The BOD POD® in the HRMU at UHCW

The investigator was trained to perform BOD POD® assessments independently. The first 10 Bod Pod assessments for COVERT-HF were supervised by a trained technician. The BOD POD® is validated and calibrated on a weekly basis by the HMRU technician to ensure

measurement accuracy. The particular room the BOD POD® is contained within is designed to minimise measurement noise and pressure disturbances.

Participants were asked not to eat/drink/exercise for at least two hours prior to the test. The 6MWT was always performed after this. The same investigator performed all the BOD POD® assessments within the Sub-Study. Participants were asked to take off their clothes down to their underwear. All participants wore lycra swim caps to flatten their hair down. These steps in optimisation minimise the participants surface area, allowing for a more accurate estimate of body composition. A calibration of the BOD POD® is run prior to every test using a 50 litre cylinder. Participant details (height, gender, age) were entered into the BOD POD® to contribute to the calculation of the body composition. The total weight was measured on the attached calibrated scales. A predicted lung volume was used in the COVERT-HF BOD POD® procedure, due to the required participant time to learn the precise breathing technique.

The participant then stepped into the chamber and the door was closed. Participants were asked to sit still and take regular breaths. A short sequence ensues where body surface area is measured. The sequence is repeated at least once and an average of the measurement is taken. When both measurements demonstrated either a $\geq$0.2% or $\geq$150ml difference, a third measurement was required.[261] Following the test the participant's waist circumference was measured, 2cm above the iliac crest line.

3.5.7.10 Blood Sampling

Participants were asked to starve for two hours and rest for one hour before blood sampling. They were asked to refrain from smoking and drinking caffeine on the morning of the procedure. Anti-platelet medication is expected to be taken by a significant proportion of the cohort and may affect the expression platelet specific miRNA.[147] No heparin is used during CRT implantation eliminating a recognised confounder for microRNA expression.[262]

3.5.7.10.1 Peripheral Venous Sampling

Peripheral blood was taken from a large peripheral vein, ideally the anterior cubital fossa vein in the right arm. A reasonable alternative was considered if this vein was not accessible. Whole blood was directly aliquoted into citrate and EDTA tubes. A total of 30ml whole blood was taken in total. Preparation and storage of samples is outlined below. Samples were taken for local laboratory analysis alongside biomarker testing.

3.5.7.10.2 Coronary Sinus (Sub-Study)

The CS is directly accessed during the CRT implantation for placement of the LV placing lead. Access is obtained with a catheter allowing blood samples to be directly taken from the venous drainage of the heart. Previous studies of ECM biomarkers has shown the potential value of directly sampled the CS both in physiological understanding, but also clinic value.[263] **Chapter 4** specifically discusses the evidence of sampling biomarkers from the CS and the information this has provided. Sampling the CS was considered to be practical and safe

(already being performed) and would add value to the understanding of ECM and miRNA behaviour

Additional permission was gained from a substantial amendment to sample CS blood during the CRT procedure, to examine variation in biomarker level in the heart and peripherally. Over half the cohort were eligible to take part in this sub-study (n=26). The CS was directly cannulated during implantation of the LV pacing lead. Following direct cannulation of the CS 30ml of blood was sampled with the first 10ml being immediately discarded. The remaining 20ml of whole blood was distributed appropriately to blood tubes as outlined in the above section. Blood was centrifuged and stored by the methods outlined below.

3.5.7.10.3 Laboratory Parameter Measurements

One citrate and EDTA blood vial from peripheral sampling was sent immediately to the UHCW local laboratory for measurements of Haemoglobin, renal function, NT-*pro*-BNP and in established diabetics HbA1C.

3.5.7.10.4 High Sensitivity Troponin-T

Following completion of the participant recruitment, discussions regarding ECM and miR biomarker analysis concluded that hs-TnT assessment would be important in novel biomarker analysis. Electrochemiluminescence Immunoassay (ECLIA) analysis was

performed at UHCW using the cobas e 602 module of the cobas ®8000 modular analyser series (Roche Diagnostics, Basel, Switzerland). The HS-TnT immune assay (cobas®, Roche Diagnostics) utilised the sandwich electrochemiluminescence (ECL) principle, which is discussed in later in this chapter. Hs-TnT was performed on frozen serum samples (-80°C). The literature and the manufacturers determine the immunoassay is stable and reliable for frozen samples with little variation in results.[264]

3.5.7.10.5 Blood Preparation and Storage

Whole blood is transferred to citrate and EDTA blood tubes immediately after being taken. Blood vials selected for biomarker analysis stood at room temperature for a minimum of 30 minutes and undergo centrifugation within maximum of an hour. Centrifugation was at 3500 rpm for 10 minutes at room temperature. Both serum and plasma was produced in separation phases. The top phase was taken from both plasma and serum and pipetted into 5x1.2ml aliquots. Samples were then stored immediately at -80°C.

3.6 LABORATORY METHODOLOGY

Material Transfer Agreements were arranged between UHCW and all recipient centres (outlined below) before sample transfer. All participant samples analysed were anonymised prior to analysis. Dr Chris McAloon performed the enzyme linked immunosorbent(ELISA) analysis and the miRNA profiling. A blinded technician/research fellow trained and assisted with both analyses. Dr Jimiao Hu assisted with the assay (ELISA) assessment and Dr Temo Bawari guided the miRNA profiling. Batch analysis was performed for the total COVERT-HF

cohort (174 samples). All individual participant samples were analysed after final follow-up within the same batch.

ELISA quantification was employed for quantification of specific ECM biomarkers (PIIINP, MMP-2, and -9) and GDF-15 within the Clinical Science Research Laboratory led by Professor Harpal Randeva, Warwick Medical School. Electrochemiluminescence immunoassay (ECL) for ECM biomarkers (PINP and collagen I C-terminal telopeptides (CTx)) was performed by the Department of Biochemistry, Royal Liverpool and Broadgreen University Hospital NHS Trust. MiRNA profiling (miR-21,-30d,-122,-133a,-210,-486) was performed in collaboration with the Cardiovascular Proteomics group, under supervision of Professor Manuel Mayr, at the British Heart Foundation Centre, King's College London.

3.6.1 Enzyme Linked Immunosorbent Assay

ELISA quantification was performed on specific ECM biomarkers (PIIINP, MMP-2 and -9) and GDF-15. The general ELISA methods employed by our group have been previously described in the literature.[265,266] GDF-15, MMP-2 and -9 utilised the DuoSet ® ELISA assays (R&D Systems Inc, Minneapolis, Minnesota, USA) and PIIINP utilised Cusabio (Wuhan, Hubei, China). The GDF-15 assay protocol had previously been used in a published cohort study.[112] All samples analysed were prepared plasma as previously outlined**.** The samples were all analysed on the first freeze-thaw cycle. All analyses were performed in duplicate. Analyses

were performed for all four assays on 96-well plates with a 6- or 7-point standard curve on each plate.

The process and principles of quantitative sandwich ELISA are outlined in **Figure 3.10** Assay-specific protocols were followed for each analysis. For the R&D protocols capture antibodies were diluted to working concentrations in Phosphate-Buffered Saline (PBS). All 96 wells of the microplate were coated overnight with 100µl of capture antibody. Following incubation three automated wash cycles were performed by the Thermo Scientific Wellwash 4 Mk 2 microplate washer (Thermo Electrical Corporation, West Chester, Pennsylvannia, USA). Each well was then blocked with 300µl of Blocking Buffer (1% Bovine Serum Albumin (BSA) in PBS) and then incubated at room temperature for one hour. A further three automated wash cycles were then performed. The PIIINP Cuasbio assay provided a pre-coated and blocked microplate as part of the kit provided by the manufacturer.

Recombinant protein in known concentrations were used for each assay to prepare a six or seven point (dependent on protocol) standard curve, using a two-fold serial dilution. A blank dilution buffer sample was used for background correction. Serial dilutions were performed using several participants' plasma samples to identify the optimum dilution for MMP-2 and PIIINP assays. For the MMP-9 and GDF-15 assays, dilutions were based upon previous reported studies. Based on these experiments, the following dilutions were used for the whole cohort: MMP-2 1:50, MMP-9 1:100 and GDF-15 1:10. PIIINP had no dilution

performed. Sample analysis was repeated if the results fell outside the serial dilution curve in duplicate.

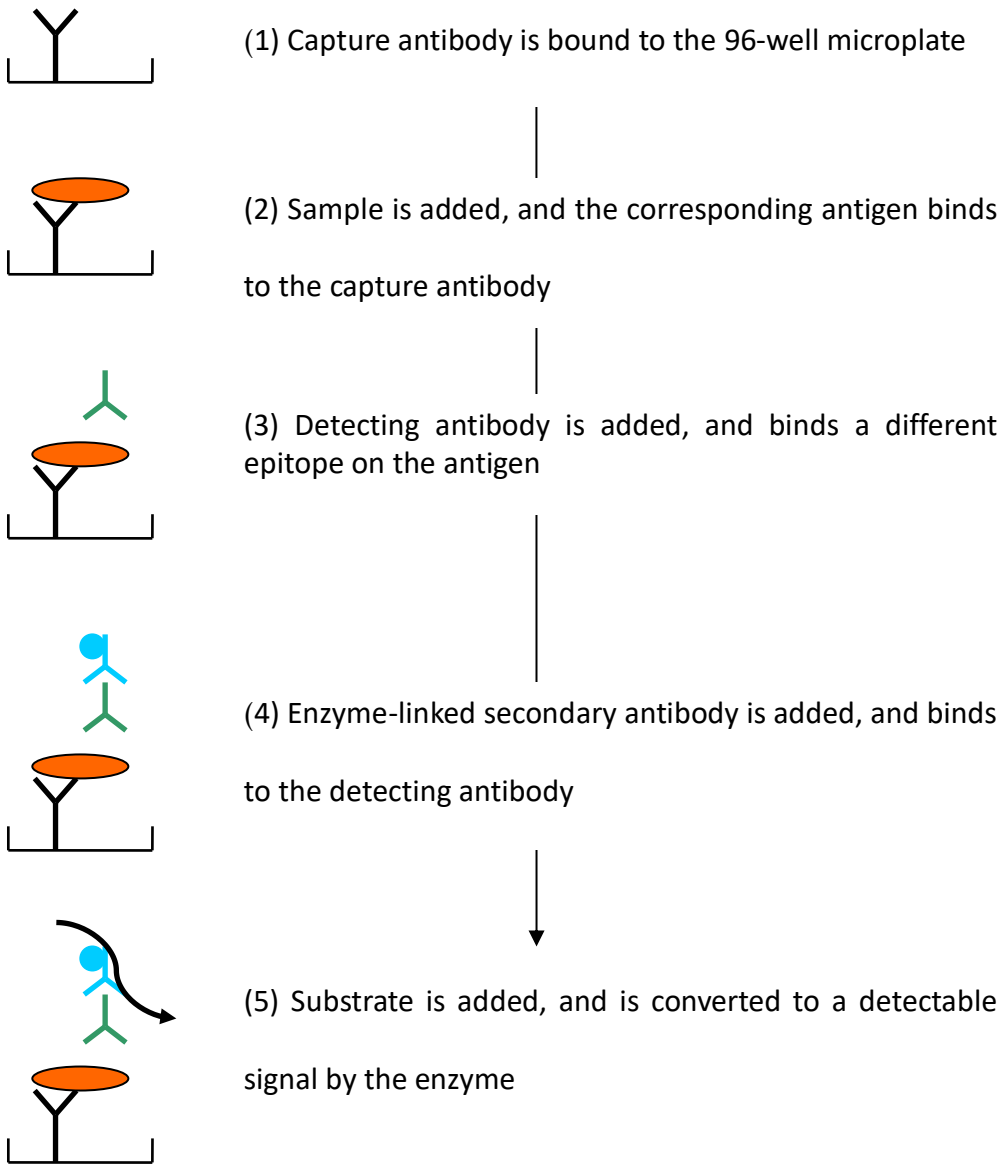


Figure 3.10 The Process of a Sandwich ELISA, Microplates are coated with a specific antibody or protein (1) to bind the corresponding protein present in the samples (2). Unbound substances are washed away, and a monoclonal antibody specific to the protein being measured is added to the plate (3). A second wash removes unbound antibody and an enzyme linked antibody specific for the primary antibody is added (4), followed by an amplifier solution which develops colour in proportion to the amount of measured substance present (5). The reaction is stopped by addition of a stop solution (usually an acid); plates are then read using a microplate reader.

Following blocking and subsequent washing, 100µl of sample or standard were pipetted to each respective well and incubated at room temperature for 2 hours (PIIINP microplates incubated at 37°C). A further three automated wash cycles was then performed (liquid removed but wash was not performed for PIIINP analysis protocol). Detection antibody (biotinylated) was diluted in reagent diluent according to manufacturer's instructions (MMP-9 analysis diluent included Normal Goat Serum) and then had 100µl added to each well. Incubation was for two hours at room temperature for all assays except for PIIINP which was for an hour at 37°C. A working dilution of Streptavidin conjugated to horseradish peroxidase (HRP) was prepared and 100µl was pipetted into each respective well after three automated washes. Incubation was then performed for 20 minutes at room temperature (PIIINP for one hour at 37°C) in the dark. A further three automated wash cycles were then performed. 100µl of substrate solution (1:1 mixture H_2O_2 and tetramethylbenzidine) were added to each well and incubated for 20 minutes at room temperature in the dark. The PIIINP assay protocol requires the addition of 90µl of tetramethylbenzidine substrate to each well and incubated for 30 minutes at 37°C, in the dark. Finally 50µl of stop solution (2N H_2SO_4) were added to each well, ensuring thorough mixing. Time between adding substrate and stop solution was dependent on observed intensity of the substrate colour. Microplate reading was performed by PHERAstar FS (BMG Labtech, Ortenberg, Germany). The microplate reader wavelength was set to 450nm. Background noise unrelated to the assay (such as optical imperfections in the plate) was corrected for by subtracting a reference measurement that was obtained at 570nm.

Quantification of the assay target concentration was calculated by plotting obtained optical density values to a standard curve. This standard curve was generated with the serially diluted assay standard following subtraction of the background noise from each well. Optical density values (see an example in **Table 3.5**) were fitted using a four-parametric logistic regression (4PL) curve-fit.[267]

Table 3.5 Serial Dilutions Optical Densities for GDF-15 Microplate 2

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Standard Curve GDF-15 (Microplate 2)		
Serial Dilution (pg/ml)	**Optical Densities**	
500	1.976	1.936
250	1.188	1.174
125	0.585	0.459
62.5	0.216	0.196
31.3	0.086	0.075
15.6	0.041	0.043
7.81	0.023	0.026

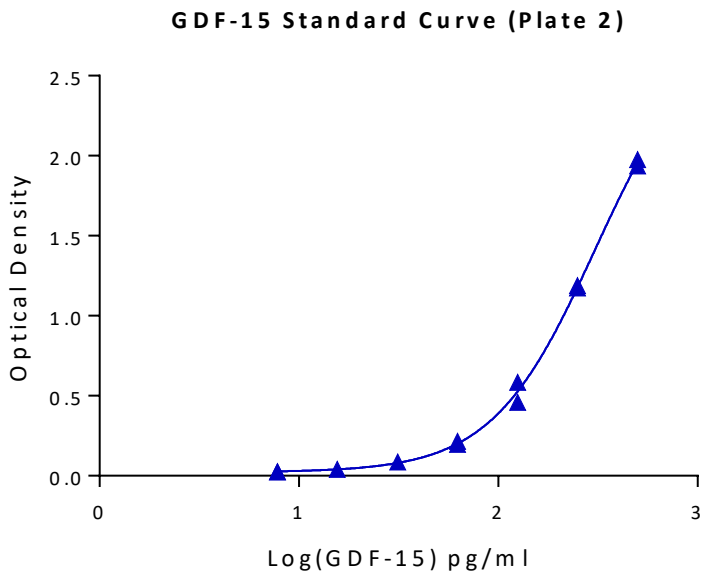
The 4PL model is the most suitable for fitting a standard curve for many complex biological systems and is the gold standard in ELISA analysis.[267] The 4PL model is summarised by the following equation, where four parameters are calculated to fit a curve to a set of standards:

$$y = d + \frac{a - d}{1 + \left(\frac{x}{c}\right)^{b}}$$

The known standard concentrations are logarithmically converted to produce a semi-logarithmic scale for fitting the 4PL model. The best-fit is calculated based upon the optical

densities of the set of standards (for example in **Table 3.5**) producing an S-shaped curve. In the equation **y** represents the response value (i.e. optical density of the standard) and the **x** represents the dose value (i.e. the known concentration of the standard), whilst **a** and **d** represent the maximum and minimum asymptotes (horizontals) of the curve respectively (and they can be interchanged). The **a** and **d** values are the same units as the **y** value. The **c** parameter represents the point of inflection (the midway point between **a** and **d**). The **b** parameter is the hill's slope of the curve (relates to steepness of curve at point **c**).

GraphPad ® PRISM 2007 version 6.0 (San Diego, California, USA) was used to calculate a 4PL curve to fit the set of standards and replicated on an online ELISA analysis tool (www.elisaanalysis.com).[268-270] A 4PL curve fit was calculated for each individual microplate. The strength of each fit was tested with the strength of the correlation and was accepted if above a coefficient of >0.97. **Figure 3.11** demonstrates an example of best-fit for GDF-15 microplate 2 from the COVERT-HF study (using the values generated in **Table 3.5**). Samples of unknown concentrations are expressed relative to the calculated standard curve. The 4 parameters calculated for this particular example is demonstrated. Reversing the 4PL curve when all 4 parameters and optical density is known allows interpolation of the unknown target concentrations. This was performed within Microsoft Excel 2010 following the 4PL calculations in GrapPad PRISM. Measured mean concentrations of duplicates underwent multiplication by the respective dilution factor (see above).



a=2.796 b=1.687 c=304.9 d=0.02211

Figure 3.11 The 4PL Best-Fit Standard Curve for GDF-15 Plate 2. Values a,b,c and d are calculated for the plotting of the known set of standard concentration and best fitting a 4PL standard curve to the values. The standard curve allowed concentrations in samples to be calculated.

The precision and reliability of the ELISA assay was estimated by calculating the inter- and intra-assay coefficient of variability (CV). The inter-assay CV is an important measure of plate-to-plate consistency. This is particularly important in large cohort studies like COVERT-HF as five microplates were required to assess one biomarker assay in all samples. The highest and lowest duplicate values are utilised to calculate high and low microplate means. These high and low microplate means were combined to calculate an average mean and standard deviation of the means, which in turn allowed for the calculation of the percentage CV of means for highest and lowest values. An average of these measures allowed

calculation of the inter-assay CV. The intra-assay CV assesses the individual variation between duplicates upon each microplate. The results are presented as a mean of all individual intra-assay CV's on one microplate. The mean concentration and the standard deviation of the duplicates were calculated. Subsequently the standard deviation was divided by the duplicate mean and the individual intra-assay CV percentage was calculated and the average for the plate calculated.

3.6.2. Electrochemiluminescence Immunoassay

The Royal Liverpool and Broadgreen University Hospital NHS Trust have extensive experience of performing this specific immunoassay (PINP and CTx) on large quantities of samples commercially and for research purposes. The samples were processed and analysed on the **Cobas e** 602 module of the Cobas®8000 modular analyser series (Roche Diagnostics, Basel, Switzerland). Immunoassays for PINP (total P1NP) and CTx (ß-CrossLaps/serum) were produced by Cobas® specifically for this analyser. The immunoassays inter and intra assay of precision for P1NP was <3.0%, and for CTx was <2.5%. The assays manufacturer calculated measures and were locally validated according to international Clinical and Laboratory Standards Institute EP05-A3 protocol.[271]

ECL is a highly sensitive process where reactive molecules are generated from stable precursors at the surface of an electrode.[272,273] The highly reactive species interact to produce light.[273,274] ECL is based around two electrochemically active substances, the ruthenium complex and tripropylamine (TPA). Both substances remain stable until a voltage

is applied. **Figure 3.12** outlines the ECL reaction that occurs between ruthenium and TPA when voltage is applied in the reaction field at the electrode.

Three test principles underpin ECLIA; competitive, sandwich and bridging principle. Both the PINP and CTx immunoassays utilise the sandwich ECLIA methodology. **Figure 3.13** outlines the process of sandwich ECLIA. Measurement of the specific antigen/analyte quantity is based upon the amount of ECL light emission compared to the established standard calibration curve. In the ECL reaction ruthenium is constantly regenerated through the reaction, leading to multiple photons release for every one immune complex present. The amplification process makes the process particularly sensitive for quantification purposes. TPA is depleted rapidly through the process and must be present is excess and depletes following peak light emission.

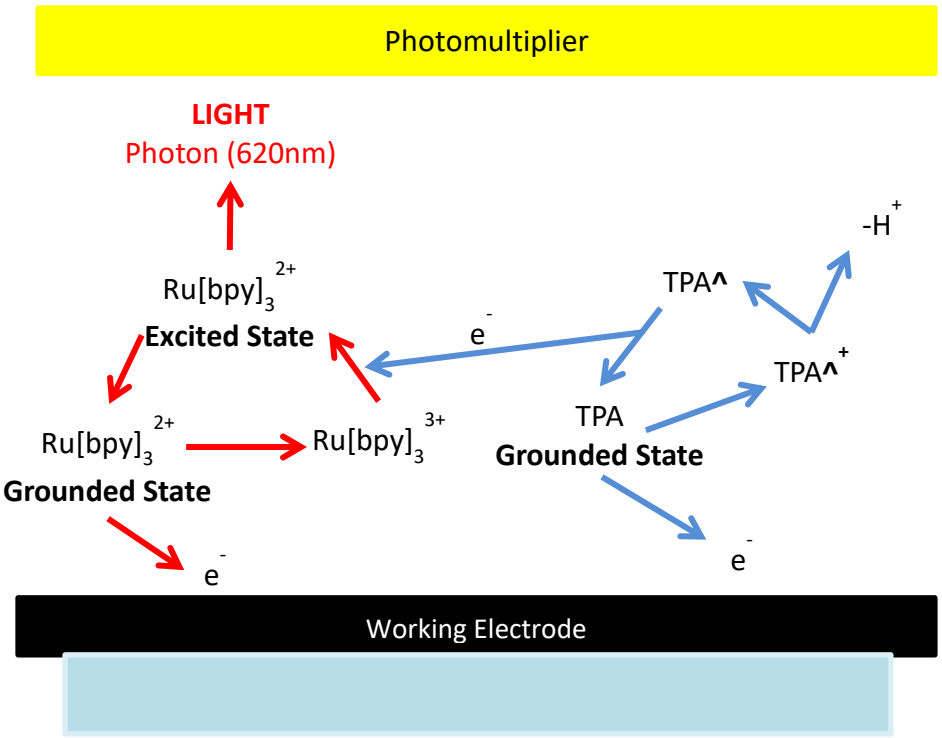
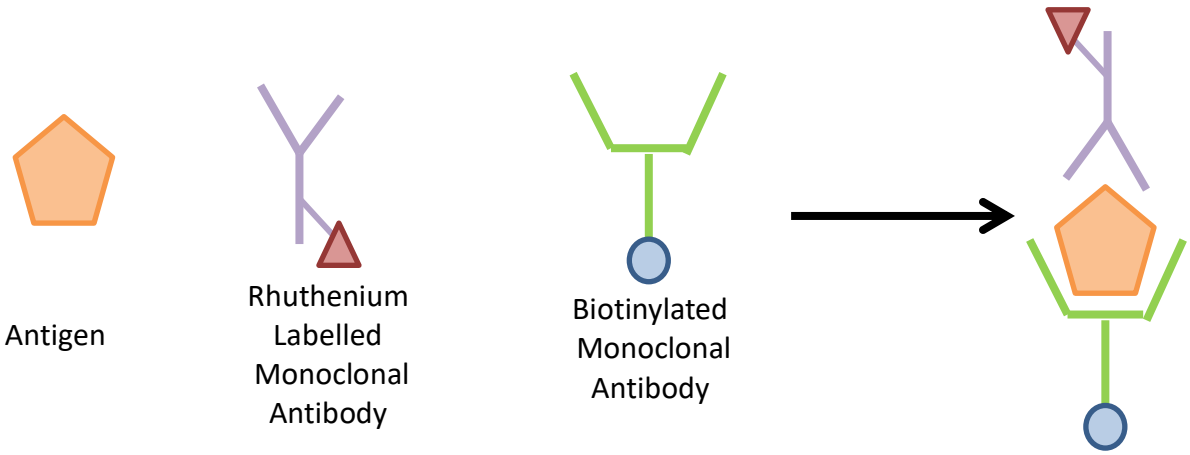
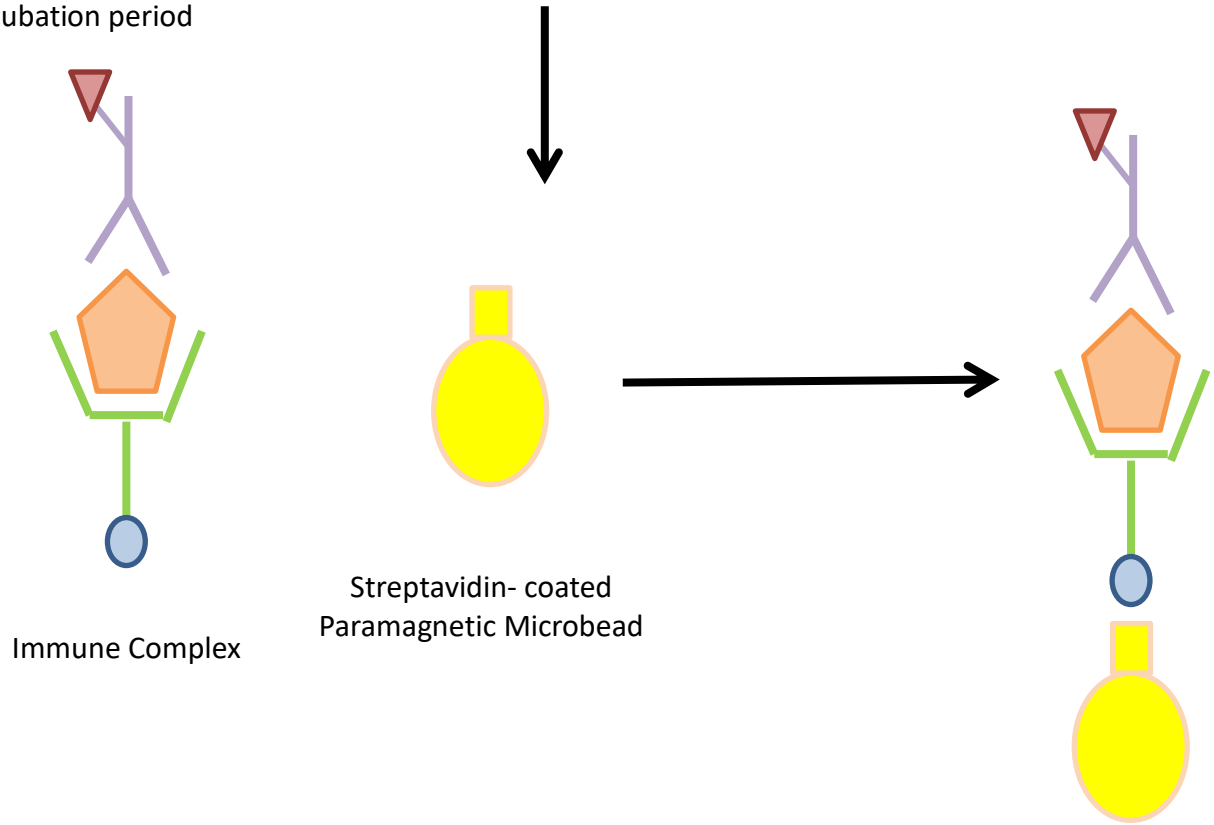


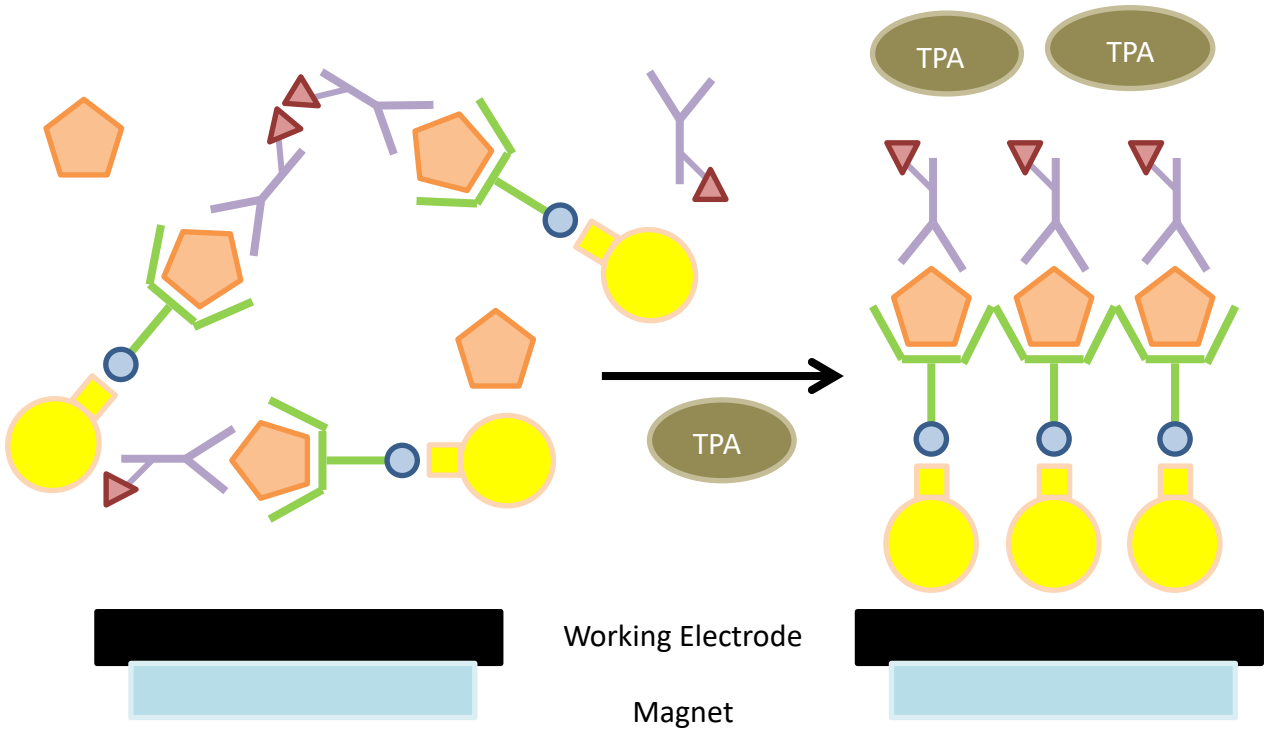
Figure3.12 The ECL Reaction occurs on the platinum electrode when voltage applied creates an electrical field and all materials presen react. TPA is oxidised at the electrode releasing an electron (e-) and forming a intermediate TPA radical cation (TPA**^**$^{+}$**)**, which further reacts by releasing a proton (H+) to form a TPA radical (TPA**^**). Simultaneously the ruthenium complex releases an electron at the surface of the electrode to form the $Ru[bpy]_3^{3+}$ cation. This cation reacts with the TPA radical, reducing $Ru[bpy]_3^{3+}$ to $Ru[bpy]_3^{2+}$ which forms an excited state due to energy transfer. This excited state is unstable and decays with the emission of a photon (620nm) its stable state. The reaction continues while TPA is abundant.



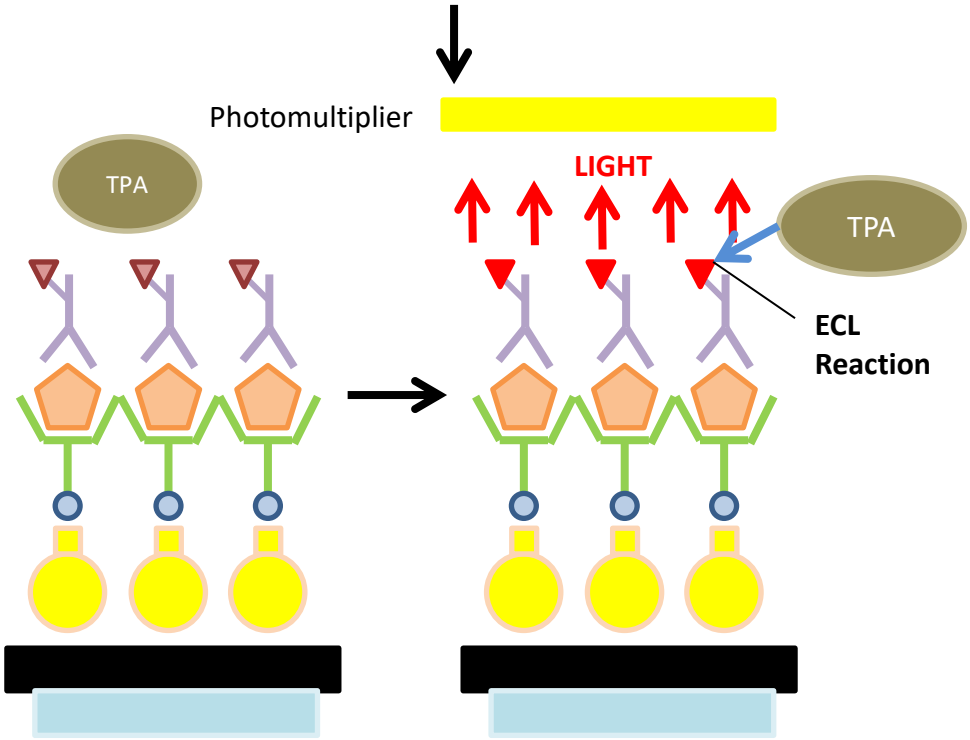
(1) Incubation of antigen, biotinylated monoclonal antibody and ruthenium complex labelled monoclonal antibody; antibodies capture the specific antigen during the incubation period



(2) Addition of strepavidin-coated paramagnetic microbeads; during incubation biotinylated antibody attaches to strepavidin



(3) Reaction mixture transported into measuring cell; immune complexes are magnetically entrapped on the working electrode; unbound reagent and sample are washed away with ProCell;TPA is simultaneously added during wash.



(4) Electrochemiluminescence reaction (ECL) of ruthenium complex (ruthenium-tri[bipyridyl)$^{2+}$ and TPA when voltage applied at the working electrode producing light; amount of light proportional to the amount of the specific antigen

Figure 3.13 The Process of sandwich ECLIA.

3.6.3 MiRNA Profiling

MiRNA profiling was performed collaboratively in the British Heart Foundation Centre, King's College London (Prof Manual Mayr). Six circulating miRNA specifically associated with cardiac adverse remodelling and ECM alteration in heart failure, alongside changes in expression with CRT implantation were selected to be measured in the COVERT-HF cohort. The specific miRNA were -21, -29b, -122, 133a, -210, and -486. **Chapter 1** specifically discusses the literature which informed these selection decisions. Profiling miRNA was performed on 174 plasma samples for the entire COVERT-HF cohort at the same time. The plasma samples are defined as Platelet-Poor Plasma (PPP).

3.6.3.1 RNA Extraction

Total ribonucleic acid was isolated from PPP samples using the miRNeasy Mini kit (Qiagen, Hilden, Germany), using phenol- and chloroform-based extraction. This previously reported RNA extraction method is discussed in detail in this section.

The 174 samples were extracted sequentially in batches of 24. Following defrosting, 500µl of PPP were transferred to 1.5 ml Eppendorf tubes and centrifuged at 4,000rpm at 4°C for 10 minutes. To minimise RNA degradation, samples were kept on ice throughout.

An exogenous miRNA (spike-in) was added at the start of the extraction process to allow for normalisation of the RNA isolation. This Spike-in is a synthetic miRNA based on the sequence of cel-miR-39-3p in the C. *Elegans* nematode. This miRNA does not occur in mammals, allowing for its use as an exogenous control. Following reconstitution in RNase-

free H_2O at a concentration of 10 µM, cel-miR-39-3p was further diluted to 2.5 nM with RNAse-free H_20 in two steps, being vortexed at each stage. A mixture was prepared to be added to the sample, combining 4 µl of diluted cel-miR-39-3p, 194.75 µl of QIAzol lysis buffer, and 1.25 µl of bacteriophage-based carrier RNA (MS2). This mixture was prepared at the start of each sample batch, allowing for a sufficient amount for that batch.

QIAzol lysis reagent is a monophasic solution of phenol and guanidine thiocyanate, designed to denature protein complexes and RNases, alongside removing most of the residual DNA and protein from the lysate by organic extraction. Under a laminar flow fume hood, 500µl QIAzol reagent was placed in each Eppendorf tube and subsequently 100µl of each samples was added, which was then vortexed, 30x inverted and incubated at room temperature for 5 minutes. Subsequently 200µl Spike/QIAzol/MS2 mixture was added to each tube, followed by being immediately vortexed, 30x inverted and incubated at room temperature for 5 minutes.

Next 140µl chloroform was added to each Eppendorf tube, followed by being vortexed, shaken for 30 seconds and incubated at room temperate for 5 minutes. The Phenol to Chloroform ratio 5:1 is established with these additions, which is the optimal condition for producing conformational changes to proteins and lipids. Chloroform addition to phenol is more efficient at denaturing proteins than either reagent is individually. Furthermore, the addition of chloroform forces a sharper separation of the organic and aqueous phase during subsequent centrifugation as it is miscible with phenol and has a higher density than phenol ($1.47g/cm^3$ vs $1.07g/cm^3$), which assists the removal of the aqueous phase with minimal

impact on the organic phase. Consequently, phenol on its own would retain 10-15% of the aqueous phase and result in a lower yield of RNA.

The pH of phenol determines the separation of RNA and DNA between the two phases. When the pH is neutral or minimally alkaline (pH7-8), the phosphate diesters in the nucleic acid are negatively charged, which results in retention of RNA and DNA in the aqueous phase. DNA is removed from the aqueous phase as the pH lowers, to a maximum effect at a pH 4.8. During transfer of DNA to the aqueous phase it dissolves, due to the negative charge in their phosphate groups being neutralised in acid by protonation. RNA despite being negatively charged remains in the aqueous phase, due to being single stranded and having exposed nitrogen bases, which allows it to form covalent bonds with hydrogen in H_2O. Altogether, acidic phenol causes retention of RNA in the aqueous phase and DNA in the organic phase; thus separating DNA from RNA. Moreover, during the centrifugation process and the subsequent separation, proteins contained within the samples separate out if they have charged domains or hydrophobic regions. These hydrophobic cores interact with the phenol causing precipitation at the interface between the two phases. The lipids in the sample dissolve in the lower organic phase.

Following incubation, the tubes containing sample, Spike/MS2 mixture, QIAzol and chloroform were centrifuged at 12,000rpm at 4°C for 15 minutes to cause phase separation. Following centrifugation, 280µl of upper phase were transferred to new Eppendorf tubes containing 480µl 100% ethanol, mixing together with a pipette. This step allows for RNA recovery by precipitating and separating it from contaminants, alongside providing

appropriate binding conditions for all RNA molecules from 18 nucelotide and above. After mixing thoroughly, the sample/ethanol mixture were added to the miRNeasy Mini spin column, which is then centrifuged at 13,000rpm for 1 minute at room temperature. Total RNA binds to the silica-membrane, a process enhanced by guanidinium. The flow-through was discarded. The next step involved washing out the phenol and remaining additional contaminants, which was performed by adding 700µl RWT buffer (buffer contents are considered proprietary information by the company and are therefore not disclosed) to the column. The columns were then centrifuged at 13,000 rpm for 1 minute at room temperature, with the flow-through being discarded. After this 500µl RPE buffer was pipetted into the column, and was centrifuged at 13,000 rpm for 1 minute at room temperature, with the follow-through being discarded. This step was repeated and followed by centrifugation for 2 minutes instead. The column was then placed in a clean collection tube and centrifuged at 15,000rpm for 1 minute at room temperature to facilitate further drying of the membrane.

The column was subsequently placed in a new Eppendorf tube and 35µl RNase-free H_20 was pipetted onto the membrane and centrifuged at 9,500 rpm for 1 minute at room temperature. This process elutes the RNA into the H_20. The tube containing the RNA were then stored at -80 ^{o}C until further use.

3.6.3.2 Reverse Transcription

Quantification of total RNA first requires its conversion into a cDNA template by a reverse transcriptase. This enzyme naturally occurs in retroviruses, where viral genome replication

requires conversion of RNA into DNA inside the host cell. The methodology for reverse transcription in extracted RNA is previously described in the literature.[147] A calibrator pool of RNA was created from 34 samples (9 patients) for normalisation of relative quantification of specific miRNA.

The Megaplex™ RT Primers used in this reaction are in two predefined pools (Human Pools A v2.1 and B v2.0, Applied Biosystems®, Darmstadt, Germany), each pool used in separate reverse transcription reactions. Each pool consists of up to 380 stem-looped reverse transcription primers, that allows simultaneous synthesis of cDNA for mature miRNAs. The stem-loop primers design overcomes the problem of the short length of the mature miRNA, which does not allow the use of conventional linear primers for PCR. **Figure 3.14** demonstrates a stem-loop RT primer and its role in the reverse transcription reaction. There are further advantages associated with stem-loop primers. Firstly the annealing of a short RT priming sequence to the 3'miRNA provides a better specificity for discriminating similar miRNAs in the reverse transcription reaction. Secondly the stem-loop RT primer prevents hybridisation of its primer to miRNA precursors, other long RNAs and genomic DNA. Thirdly the base stacking of the stem enhances the thermal stability of the miRNA-DNA heteroduplex, further improving the reverse transcription efficiency for short primers.

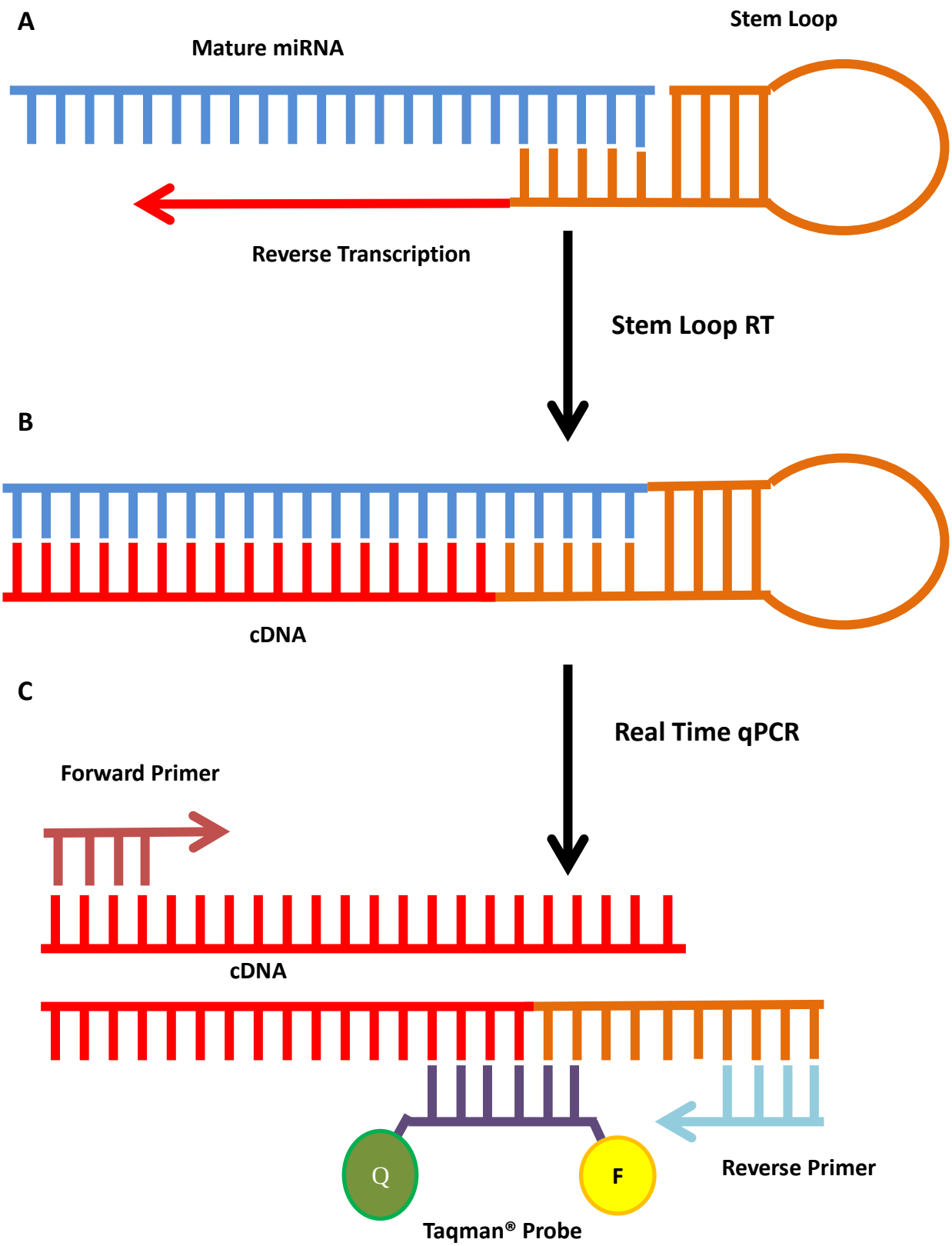

A
Mature miRNA
Stem Loop
Reverse Transcription
Stem Loop RT
B
cDNA
C
Real Time qPCR
Forward Primer
cDNA
Q
F
Reverse Primer
Taqman® Probe

Figure 3.14 MiRNA Detection and Quantification. (**A**) A stem-loop RT primer includes a 3' overhang sequence, a stem and a loop. The 3' overhang is a short sequence ranging from 5 to 8 nucleotides that is specific to the 3' end of the mature miRNA and when it binds increases the mature miRNA molecule size and adds a universal 3' priming site for subsequent qPCR.(**B)** Reverse Transcriptase used the stem-loop primers to produce DNA, complementary to the mature miRNA single strand (cDNA). Pre-amplification (not represented on this schematic) is then performed, resulting in increased quantity of the targeted cDNA prior to qPCR. (**C)** In addition to forward and reverse primers, Taqman ® qPCR assays include a probe that has high affinity for the individual target, has a fluorescent reporter (F) and quencher (Q) attached to the probe. The quencher inhibits the omission of fluorescence from the reporter. A forward and reverse primer allows DNA polymerase to produce a complementary DNA strand from the target cDNA template. In the presence of DNA polymerase the TaqMan probe is hydrolysed, releasing the quencher and allowing the emission of fluorescence. As more PCR product accumulates exponentially with repeated qPCR cycles, the fluorescence emission increases, allowing for optical detection. (Adapted [275])

An RT MasterMix was prepared for each RT reaction; 1µl 10x RT Buffer, 0.3µl 100 mM deoxynucleoside triphosphates (dNTPs) with deoxythymidine triphosphates (dTTPs) (required for DNA synthesis), 1.2µl 25 mM $MgCl_2$, 0.2 µl RNase Inhibitor, 1µl 10x Megaplex RT Primers (Pool A or B), 1.3µl 5x Cel-miR-39-3p Primer (which is not included in the Megaplex pools) and 2µl Multiscribe Reverse Transcriptase enzyme. The Applied Biosystems® Multiscribe™ is a recombinant reverse transcriptase from the Moloney murine leukaemia virus. Enough RT MasterMix was prepared for two 96-well plates. Using an Automated Liquid Handling Platform (Agilent, California, USA), 7µl of RT MasterMix was added to each well, then 3µl of each RNA sample was added to each corresponding well by hand, and mixed thoroughly. A calibrator sample (consisting of a pool of 34 samples) was loaded to designated wells on both 96-well plates. The RT-PCR reaction was performed in a Veriti Thermal Cycler (Applied Biosystems®) with settings as follows: forty cycles of 16°C for 2 min, 42°C for 1 min and 50°C for 1 sec, followed by incubation at 85°C for 5 min to terminate the reaction.

3.6.3.3 Pre-Amplification

Pre-amplification is performed immediately after RT to increase the quantity of miRNA cDNA analysis before real-time quantitative PCR. The methods utilised for pre-amplification have been previously described and validated.[276,277] The RT reaction products were further amplified using the Megaplex™ PreAmp Primers (Primers A v2.1 and B v2.0, Life Technologies, Massachusetts, USA). The Megaplex™ PreAmp Primers pools are miRNA-specific forward and reverse primers that correspond to the composition of the Megaplex RT pools, which allow unbiased pre-amplification.

A pre-amplification Mastermix was prepared for each reaction; 3µl Nuclease-free H_2O, 5µl 2x TaqMan® (Applied Biosystems®) PreAmp MasterMix and 1µl 10x Megaplex™ PreAmp Primers. Enough Pre-amplification MasterMix was prepared for two 96-well plates. The Automated Liquid Handling Platform added 9µl of pre-amplification MasterMix to each well, then 1µl RT product sample was manually added to corresponding wells, and mixed thoroughly. Calibrator RNA was loaded to the designated wells on both 96-well plates (**Figure 6.1**). The pre-amplification reaction was performed in a Veriti Thermal Cycler with the settings; 95°C for 10 min, followed by 12 cycles of 95°C for 15 sec and 60°C for 4 min. Finally, samples were heated at 95°C for 10 min to ensure enzyme inactivation. Pre-amplification reaction products were diluted with nuclease-free H_2O to a final volume of 40µl and stored at -80°C. Prior to freezing the calibrator samples were aspirated from their respective wells, pooled together, mixed, and added back to the respective wells.

3.6.3.4 Real Time Quantitative PCR

The polymerase chain reaction amplifies a segment of DNA or cDNA between regions of a known nucleotide sequence. Two oligonucleotides are used as primers (forward and reverse primers), which are different in sequence and complementary to the sequence on the opposite sides of the DNA template flanking the segment of interest. This allows amplification of the area of interest which lies between the primers. **Figure 3.15** schematically highlights the stages of a qPCR reaction. TaqMan ® miRNA assay (Applied Biosystems) was used to determine the specific expression of miR-21,-30d,-122,-133a,-210,-

486 and cel-miR-39-3p. TaqMan ® miRNA assays are highly sensitive and have a high affinity, due to the size of miRNA.

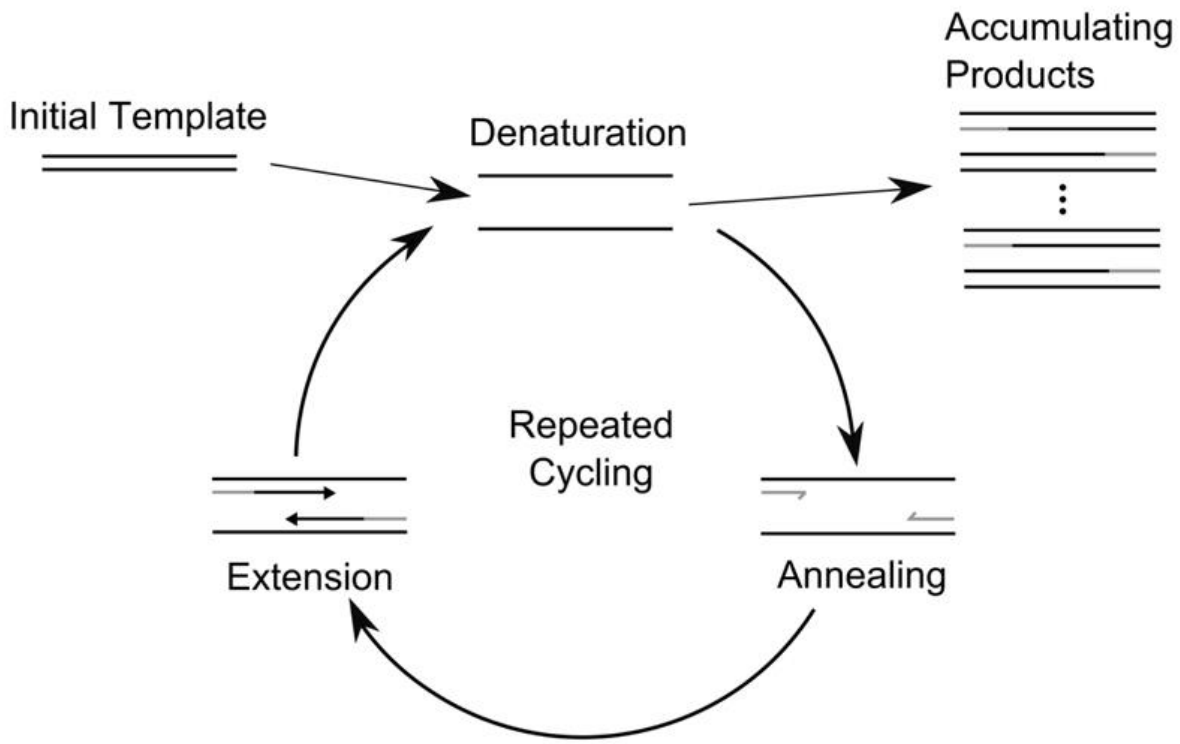


Figure 3.15 The Polymerase Chain Reaction Stages. Initially cDNA template is denatured by heating. The reaction is then cooled to allow for annealing of the two oligonucleotide primers to its complementary sequence on the targeted cDNA. A polymerase then extends downstream from the targeted template sequence. The cycle is repeated 20-40 times through successive steps of denaturation, annealing and extension. Eventually, the concentration of accumulating products becomes high enough that double strand product formation is favoured over primer annealing, and the reaction plateaus. (Adapted[278]).

A Mastermix for TaqMan® qPCR was prepared for each reaction, consisting of 2.5 µl TaqMan® PCR MasterMix and 0.25 µl TaqMan Assay. Separate Master mixes were prepared for each TaqMan® assay. Six MicroAmp Optical 384-well reaction plates (Applied

Biosystems) were mapped for every sample/assay to be performed for each specific miRNA target. Pre-amplified cDNA samples was further diluted by combining 5 µl of template with 85 µl of nuclease-free H_2O. There was 2.75µl MasterMix and 2.25µl diluted pre-amplification product added to each well by the Bravo Automated Liquid Handling Platform (Agilent). Calibrator sample/assay were mounted on each 384-well plate. The qPCR reaction was performed on a Viia 7 Real-Time PCR System (Applied Biosystems), set to run as follows: incubation at 95°C for 10 min for polymerase activation, followed by forty cycles of 95°C for 15 sec and 60°C for 1 min.

Data was analysed using ViiA 7 Software (Applied Biosystems). Relative amounts of the targets were calculated using the 2-ΔΔCT method.[276,277] Cel-miR-39-3p and the calibrator sample were utilised for normalisation. If the qPCR software reported undetermined values for individual replicates, a cycle threshold (Ct) value of 40 was assumed.

3.7 DATA MANAGEMENT

3.7.1 Study I

A procedures list of all CRT implantations between January 2009 and December 2013 was generated formed the basis of the anonymous data collection. The list was generated from the procedure database (Carddas, GE Healthcare, Horten, Norway). Once CRT procedures were excluded (**Figure 5.4**) an anonymous database was created. A Microsoft Excel 2010 (Version 14.0) database was designed to capture patient and procedure information. Specific columns had validated data entries, with pre-set options available to enter into a

particular cell. This occurred for binary and dichotomous variables. Where continuous data was collected this was limited to numeric data options.

The database was password protected. Data collection adhered to the principles of the Data Protection Act 1998 and satisfied the local Caldecott Guardians. Personal information was anonymised upon collection. Patient information and anonymised data were stored on a secure Hospital hard-drive. An encrypted USB memory stick was used to store anonymised data.

Data collection was undertaken by reviewing electronic and paper case notes. Two independent data reviews were performed to ensure high quality data. Hospital coding data on medical background and outcomes were sought. Ejection fraction and QRS duration were measured on all available pre-procedure echocardiograms and resting 12-lead ECGs to independently standardise measurements and minimise missing data. All information available was collected for each anonymised patient research record.

3.7.2 Study II

Each participant was given a unique study number to cross reference the data collected with investigations performed, alongside biological samples stored. The original database contained patient identifiable information for research study activities including scheduling visits and tracking outcome measures

Prospective data collection forms (**Appendix K**) alongside the echocardiogram reports were utilised as source documentation for inputting of the data into the database**.** The database contains all the variables recorded in the COVERT-HF study design (**Appendix J**). The database was designed to have validated entries for binary or dichotomous variables only, preventing alternative data being inputted. The database was divided into multiple worksheets (i.e. clinical assessment and medications) to allow ease of navigation and analysis. Cardiovascular outcome (MACE) was confirmed with the source data, it was entered in the COVERT-HF database.

Transthoracic echocardiograms reports and images were saved in the storage and analysis system (EchoPac, GE Healthcare, Horten, Norway). Reports were generated for each participant research visit and linked to the their clinical record.

3.7.2.1 Study II Data Quality Check

Prior to database lock and subsequent statistical analysis all source and electronic data was internally audited by a clinical trials officer to ensure accurate data entry. Where discrepancies were found errors were corrected if the mistake was obvious. When data errors were unclear, a verification conversation with the investigator was undertaken to ensure data integrity data.

3.8 STATISTICAL ANALYSIS

This section discusses the general approaches to handling the datasets and performance of statistical tests. Two specific datasets are presented in this thesis and specific statistical

methodological approaches for each dataset and analysis are discussed in the respective chapters. Clinical data was inputted into a purpose built Microsoft Excel Version 10 database, which was discussed in chapter 2. The statistical analysis was planned and verified by Dr Thomas Hamborg (Division of Health Sciences Statistics and Epidemiology, Warwick Medical School, University of Warwick). Towards the end of the PhD Prof Alan Nevile (University of Wolverhampton) kindly provided statitcical support, specifically for the prospective study. All statistical analyses were performed using Statistical Package for Social Sciences [SPSS], version 22.0 (IBM, Chicago, Illinois, USA). All figures and graphs were produced using one or in combination of GraphPad® PRISM 2007 version 6.0 (San Diego, California, USA) or SPSS. Statistically significant results for both datasets is if the p-value ≤ 0.05.

3.8.1 Database

Databases following completion of initial data entry underwent a quality check process (**chapter 5, section 5.6**) to ensure a complete data cleaning process. After the quality check the datasets underwent datalock. Following data inputting quality checking the data for both respective datasets are transferred to the respective SPSS databases prior to performance of any analysis. Each participant is given a unique study number for the statistical database that provides complete anonymity. Continuous (scale) data was transferred unadjusted. Categorical (nominal and ordinal) was consistently numerically coded to allow accurate data handling with SPSS. For example for a recorded co-morbidity variable like previous myocardial, binary data is recorded (Yes or No). For allocation to the

statistical database in SPSS a specific coding system is employed; No=0 and Yes=1. Consistent coding was applied to both databases.

3.8.2 Missing Data

Missing data is common in clinical research, especially in time sensitive, resource-intensive or longitudinal observational data collection methods.[245] Wood *et al*,[279] in 2001 showed 88.7% (63/71) trials reported partly missing data of which 17.8% had over 20% missing outcome data.[279] Missing data itself and how it is handled can create biased results, decrease study power or lead to underestimates of uncertainty, all of which can reduce the chances of drawing valid conclusions.[245] The causes of missing data (called 'censoring') can in itself be informative, when its absence may indicate something about the variable. For example a patient not attending follow-up due to travelling may be an indication of deterioration of health. The first step required to assess missing data is to establish a pattern of 'missingness' of which three patterns have been described; missing completely at random (MCAR), missing at random (MAR) and missing not at random (MNAR).[245] MCAR means the probability of data being missing is completely unrelated to all observed and unobserved characteristics. MAR does not assume patients data with missing values are related to those with complete date, rather it assumes that observed values can help explain which are missing and can help predict what they might be.[245] MAR is assumed to be a more likely mechanism of missingness than MCAR.[245] Most studies plan their techniques for data handling on this basis.[245] MNAR is the most problematic missing data pattern and is

dependent on unobserved or unknown factors. This mechanism is almost impossible to correct for.[245]

Techniques are available within statistical software (including SPSS) to assess the pattern of missing data. The assessment of missing data allows the possible pattern of missingness to be established. Often this is assumed to be an MAR pattern, as MACR is often implausible and MNAR would mean nothing can specifically be done. Assessing the pattern of missing data within the datasets allowed the handling techniques to be implemented prior to analysis.

Missing data handling strategies then need to be decided and implemented, the options include; complete case, last observation carried forward (LOCF), worst case imputation and multiple imputation.[245,279] Complete case analysis is performed only on those subject with complete data (that is being tested), this method is only valid under MCAR.[279] LOCF imputes missing values with the individual's last observation, this is rarely realistic and often conservative.[279] Worst case imputation fills all missing values with the worst case value, which leads to extreme results and is often biased. Multiple (or regression) imputation predicts the missing values from the observed individuals data.[279] This mechanism assumes observed individual data can explain the missing data and is valid for MAR mechanism of missing data.[279] Single imputation under-estimates the standard error, but is corrected by multiple imputations.[279] Missing data handling strategy for each study within this thesis was implemented following review of the censored values. The analysis of the CRT registry concluded there was a combination of MAR and MCAR. The data handling approach first

adopted a complete case analysis. The degree of missing data was anticipated and recognised. A multiple imputation regression method was utilised to handle the missing data and was compared with the complete case analysis approach (**Chapter 8**). The COVERT-HF study had less missing data observed due to the prospective study design and mechanisms to prevent it. MCAR of patterns of censoring were present, due in one case to post-procedural exclusions as failure of their CRT devices were due to complications not the individual not responding. A complete case analysis was undertaken as the assessment strategy.

3.8.3 Descriptive and Inferential Data Analysis

Understanding what the data collected represents is critical to the analysis. Data was identified as continuous (scale) or categorical (nominal or ordinal). Continuous data requires assessment of distribution for decisions on its handling for statistical testing. Outcome and potential predictor data variables were handled in an identical manner during the initial analysis. This next section described those initial steps in the analysis of both datasets before performing the more comprehensive statistical testing.

3.8.3.1 Descriptive Statistics

Categorical variables were reported as frequency and percentages. Continuous data prior to presentation underwent assessment of distribution (described in the next section). Normally distributed data were reported as mean ± standard deviation (SD). Non-normally

distributed data were reported as median (range). Specific consideration to the handling of NYHA symptom classification was given. NYHA symptom classification is an ordinal scale and was presented as a categorical variable and comparative assessment performed.

3.8.3.2 Distribution of Continuous Data

The first step in assessment of the continuous variables in both datasets was the distribution. This was a critical step to know how to present the data, which variables require transformation (discussed later in this section) on and knowing comparative or correlation statistical tests to perform. Initial graphical assessment of variable data distribution is performed using a histogram. For a normal (Gaussian) distribution, a histogram will demonstrate a bell shaped curve. **Figure 3.16** demonstrates examples from the COVERT-HF study of normal and non-normal distributed variables. An additional graphical assessment that was performed when histograms were challenging to interpret were normal probability plots or Quantile-Quantile (Q-Q) plots. For a Q-Q plot, an expected normal distribution is plotted against the actual distribution. When the observed datapoints fall on the straight line, good adherence to the expected data is demonstrated. **Figure 3.17** demonstrated examples of Q-Q plots for normal and non-normally distributed data for the same variables demonstrated in **Figure 3.16** histograms. Following graphical plotting the tests of normality were undertaken on the continuous variable. Specifically the Kolmogorov-Smirnov test was performed, which assesses the normality of the distribution of scores. Approximate normality is assumed if a non-significant p-value is returned.[280] Assigning data distribution conclusion is based on statistical and graphical assessment.

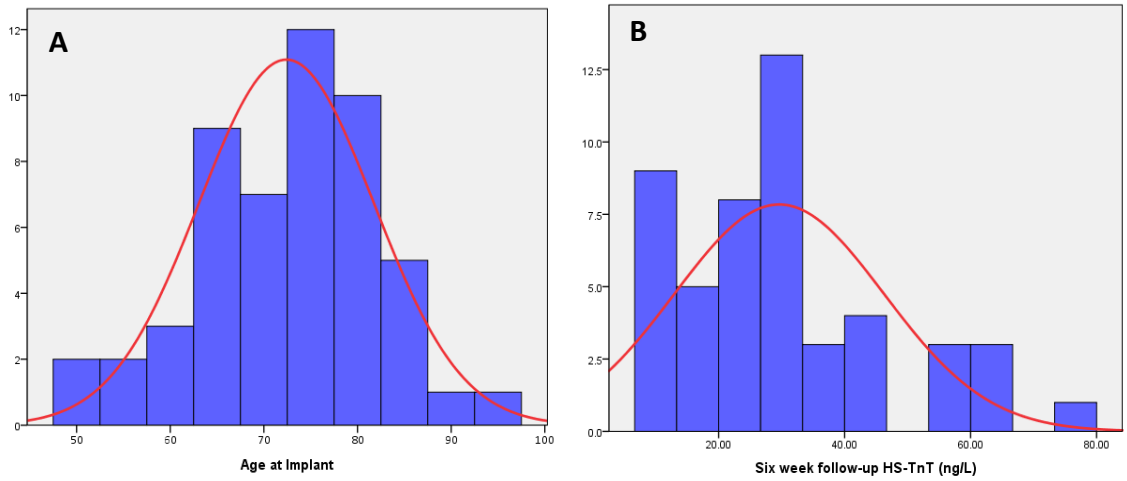


Figure 3.16 Examples of Histograms. Illustrations both taken from the COVERT-HF dataset. (**A**) Shows normal distribution of the variable '*Age at* Implant' which is demonstrated by the bell shaped curve. (**B**) Shows non-normal distribution of the variable 'HS-TnT' at six weeks follow-up, which is demonstrated by a positive skew.

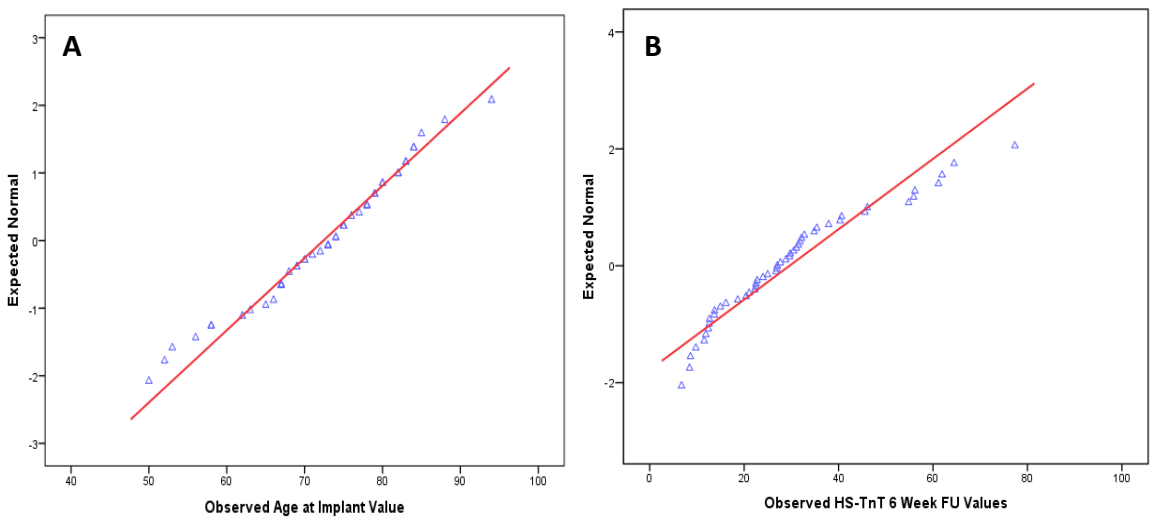


Figure 3.17 Examples of Quantile-Quantile Plots. Illustrations both taken from the COVERT-HF dataset. The same variables are used as in **Figure 7.1**, for means of comparison (**A**) Shows normal distribution of the variable '*Age at* Implant' which observed variables falling approximately on a straight line. (**B**) Shows non-distribution of the variable 'HS-TnT' at six weeks follow-up, where observed data points do not fit well with the expected data line.

3.8.3.3 Outlier Variables

Outlier values were assessed for within each cohort as many statistical processes are sensitive to their presence. The potential influence of outlier variables was potentially problematic within the COVERT-HF cohort due to the size of the study. Extreme outliers were suggested on histograms (**Figure 7.1**) where extreme values were found in the tails on their own. Subsequently Boxplots were created to examine the distribution of data and examine outlier values. Each distribution in a Boxplot is represented by a box and whiskers. The length of the box is the inter-quartile range and contains fifty percent of the data. The whiskers contain the lowest and highest values. Boxplot extreme outlier values are present if they are $\geq$1.5x the box lengths from the edge of the box.[280] Outlier variables are represented as individual data-points. **Figure 3.18** demonstrates the actual Boxplot distribution for NT-*pro*-BNP at baseline as an example, which has two extreme outlier results.

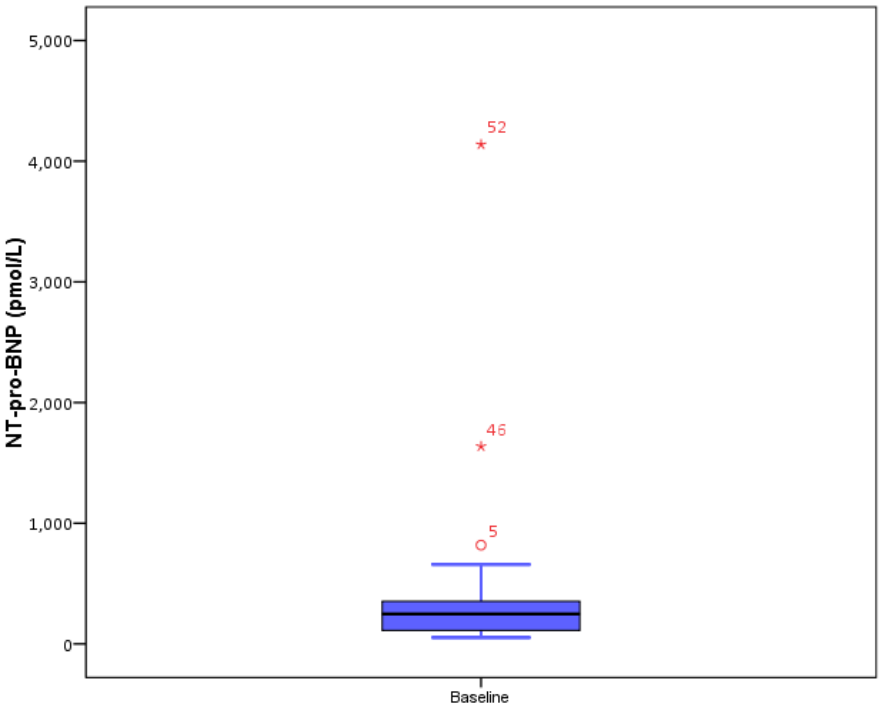


Figure 3.18 A Boxplot of Baseline NT-*pro*-BNP (pmol/L). Illustration of a variable with outliers from the COVERT-HF dataset. The three outlier variable were labelled individually with extreme values being asterixed.

3.8.3.4 Data Cleaning

Evaluation of outlier results is undertaken to examine for the reason for the value. If an error is identified then the value was removed or altered. During the data quality process the first aspect of the data cleaning was performed, removing erroneous variables etc. When no error was apparent evaluation was performed as to the explanation for the value and whether it needed to be removed. For example NT-pro-BNP baseline levels identified two extreme values and these were not due to errors (**Figure 7.3**). These values were

correct and clinically expected for both participants, furthermore follow-up levels were similar. In this instance the values reflect the patient and remained in the dataset.

3.8.3.5 Transformation

The distribution of continuous data was assessed for normality. Non-normally distributed continuous data cannot be assessed using parametric statistical tests. Transformation were performed to normalise non-normally distributed continuous data variables, to allow analysis with a parametric statistical test as part of the logistic regression model in the COVERT-HF study. Logarithmic conversion was considered for ECM biomarkers, NT-pro-BNP, GDF-15 and hs-TnT expression data if their distribution was non-normal and demonstrated a positive skew pattern. **Figure 3.16 (B)** demonstrates an example of a positive skew distribution. Transformation was attempted on non-normally biomarker expression data to allow easier comparison with the miRNA relative quantification data in the statistical models. Performing logarithmic conversion can sometimes convert non-normally distributed data to a normal distribution, which allows analysis via parametric methods to be undertaken. Transformed data was examined for their normality of distribution. Where transformation meant the continuous variable was now normally distributed was that transformed variable used in the logistic regression prediction model.

3.8.3.6 Comparisons between Groups

Grouping of the COVERT-HF cohort was performed for outcome measures. The principle grouping of the cohort was performed on the basis of functional response status (primary outcome). Further grouping was performed for echocardiographic response and MACE status. Moreover changes in variables were considered across all three research visits and by the functional response grouping.

Comparison between groups for categorical data was performed using the Chi-Squared test for independence. When the minimum expected cell frequency was not achieved the Fisher's Exact test was performed as the best alternative. Normally distributed continuous data used the independent t-test for unpaired cohort comparisons. The non-parametric equivalent test used for unpaired comparisons of non-normally distributed variables was the Mann-Whitney U test. Paired comparison occurred for variables that were measured in the same partipant at the same time, for example within the COVERT-HF study; peripheral vs CS baseline biomarker expression. A paired t-test or Wilcoxon Signed Rank Test was used for normally and non-normally distributed data respectively.

Assessment of repeated measures (>2) was required within the analysis of the COVERT-HF study for continuous data variables that were measured at all participant study visits. One way repeated measures analysis of variance (ANOVA) was performed on normally distributed data. For non-normally distributed variables Friedman's Test was utilised.

3.8.3.7 Correlation Analysis

Relationships between two continuous variables are explored using a bivariate correlation analysis. The correlation analysis conducted assesses the strength and direction of the linear association between two continuous variables.[281] Initial evaluation of the association was with a simple scatterplot, which provided an eyeball assessment of the association. Several correlation estimators are available dependent on the variables distribution.[280] Two important measures that have been used for the COVERT-HF dataset are Pearson correlation (parametric) and Spearman Rank Order correlation (non-parametric). Both analyses provide a correlation coefficient (r), which is a calculated ratio of covariance between the two variables.[280,281] The correlation coefficients (r) can only take on values between -1 and +1 to describe the linear relation between the two variables. A value of +1 described a perfect positive linear relationship, whereas -1 demonstrates a perfect negative linear relationship.[280,281] Categorisation of the strength of the relationship is estimated to be; small (r=0.10 – 0.29), medium (r=0.30 – 0.49) and large (r=0.50 – 1.0).[280] **Figure 3.19** shows scatterplot with a line of best-fit and a calculated correlation coefficient (Pearson's), which demonstrated a strong negative linear relationship for the COVERT-HF dataset.

Correlation analysis was performed on the COVERT-HF dataset to explore relationship between two variables. Associations between continuous functional, echocardiographic, biomarkers and body composition variables at baseline and following differences following CRT implantation were explored. Strengths and direction of linear associations were reported.

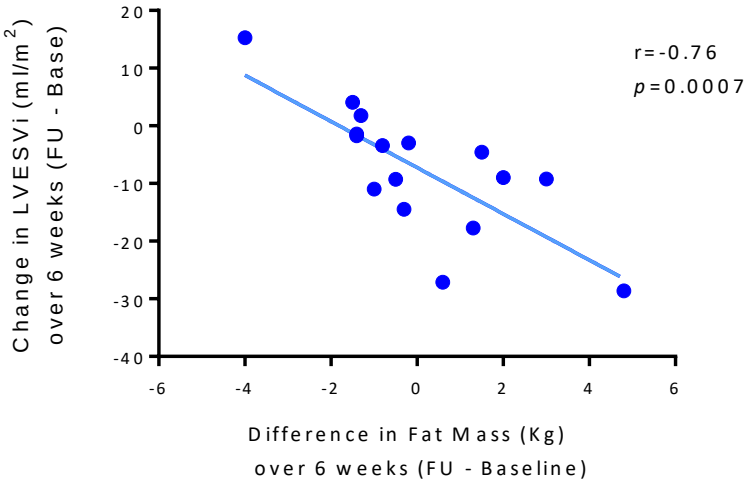


Figure 3.19 Scatterplot of change in LVESVi and fat mass over 6 Weeks. Illustrated from the COVERT-HF study

3.8.4 Mixed Between-Within Subjects ANOVA

The analysis of variance can be approached with a between-subject design comparing ≥2 different groups) and a with in or repeated measures design (one group exposed to ≥2 conditions).[280] In certain situations these approaches are required to be combined when there are two independent variables (between-subject and within-subject) present that will affect the dependent variable. In the COVERT-HF study the variation in biomarkers expression, echocardiographic LV geometry and functional parameters (dependent variable) through the observation (within-subject factor) period was required to understand the impact of CRT following its implantation. Moreover, understanding the pattern of variation between responders and non-responders (between-subject factor) over the time of the observation period (within-subject variable) is important. A mixed between-within subjects ANOVA allows both independent variables (between-subject and within subject) impact on

the dependent variable to be analysed. The first step during this analysis was to test for homogeneity of variances (sphericity) for each combination of the two independent variables. The sphericity (measures variation) of the differences between all groups of the independent variables should be equal. The interaction effect was the next aspect of the analysis performed, which tests the impact of each independent variable on each other. Assuming no significant interaction the main effects were then interpreted for the with-in subject and between-subject variables statistical differences. The effect size was estimated by the Partial Eta Squared Value.[280]

3.8.5 Logistic Regression

Binary logistic regression was performed in both datasets to test the pre-selected potential predictors (independent variables) ability to predict CRT response (binary categorical outcome variable). Logistic regression requires the dependent outcome variable to be categorical. There are several logistic regression techniques available dependent on the number of outcome groups. The predictors can be continuous or categorical or a mixture of both. The logistic regression calculated an odds ratio for a particular independent variable estimating its ability to predict the outcome.

Univariate logistic regression analysis was performed initially to examine the relationship between the potential predictor and the outcome. The relationship demonstrated on univariate analysis may not reflect the effect of the potential predictor as an explanatory

variable on the outcome. It may reflect the effect of another confounder variable. A potential predictor variable was entered into the multivariate logistic regression model when the p-value on univariate analysis was <0.15. A high p-value threshold was set to allow inclusion of all variables that might show an effect in the multivariate analysis which might be excluded with a lower threshold due to the size of the cohort (to retain potentially important variables).[282] Multivariate modelling utilises multiple potential-predictor variables to explain the variation in the outcome variable. The model aims to identify the strongest combination of predictor variable which predict the particular outcome. The final model is established when all the predictor variables remaining in the model have a $p<0.05$. Once this is achieved a multiple variable equation is created that allows calculation for the odds of predicting the outcome probability if all the identified strong predictors are present. Forward selection and backward elimination techniques were performed for multivariate binary logistic regression to test the strength and reproducibility of the predictors. A chi-square statistic of the Hosmer-Lemeshow tests compared the observed frequencies with those anticipated in a linear model. A non-significant result was suggestive that the model was well attuned.

3.8.6 Survival Analysis

3.8.6.1 Life Tables and Kaplan Meier Survival Curves

Survival analysis is broadly categorised as a set of methods used to analyse the time required for an event of interest to occur. An event is whatever the study design has designated an end-point (e.g. MACE) which is normally a terminal event. The time-to-event

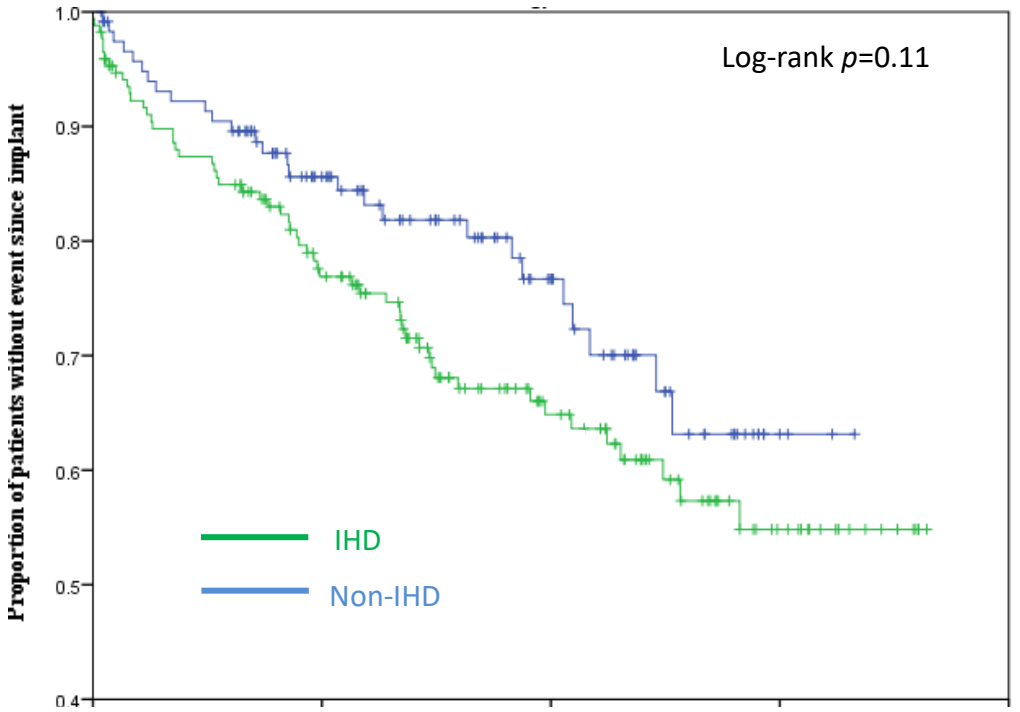
is the critical variable that is recorded and observed. Survival analysis techniques account for the events or uncensored observations.[283] Importantly survival analysis also accounts for those observations where events do not occur in the study period or a participant leaves the study early for an unrelated reason (called censoring).[283] Censoring is unrelated to the unobserved times to the event. Survival analysis unlike linear regression for instance can account for those participants with censored observations.[283] The life tables and Kaplan-Meier curves are simple statistical procedures that account for censoring in comparison of groups.[283]

The life table's method involves the total study observation period being dividied into fixed intervals of time. **Figure 3.20** demonstrates a simplistic life table as part of a survival analysis performed as part of the CRT registry analysis. The number of patients at risk of cardiovascular outcome (e.g. MACE) occurring was listed at the start of the study and then listed at each subsequent time interval until the end of the study. For the CRT registry study that was in 20 month intervals. Both groups (e.g. aetiology) were compared against each other at all intervals. Cumulative survival was calculated for each subsequent interval. The endpoint or event must be defined prior to the analysis, in **figure 7.5** the event is MACE.

The Kaplan-Meier survival curve method estimates and graphs survival probability as a function of time. The curves demonstrate the stepwise changes in cumulative survival (**figure 7.5**). To test the overall differences in the estimated survival curves for two (or more) groups the non-parametric log-rank test can be performed. Importantly the log-rank

test cannot discriminate or explore the influence of multiple independent variables (potential predictors) simultaneously.[283] Crucially the Kaplan-Meier analysis accounts for whole curve and not isolated datapoints.[283] To study the impact of multiple independent variables on the time-to-event outcome, the Cox proportional regression model was employed.

In the CRT registry the survival analysis employed life-tables and Kaplan-Meier curves to estimate survival (time-to-event) for the potential binary predictors (independent predictors) for cardiovascular outcomes. These non-parametric techniques offered the most robust techniques of estimating survival rates given the potential burden of censoring (for example the loss to follow-up of those patients returning to local secondary care services and the variation in observation period for each participant). The log-rank test was used to examine univariate difference between two groups but would not account for other confounders, therefore a Cox proportional hazards regression was performed.



Month	0	20	40	60	80
No IHD	119	76	37	3	
IHD	171	111	55	17	

Figure 3.20 Kaplan-Meier survival curves for the CRT retrospective registry (n=300) from MACE for HF aetiology. Life tables are given in the table at the bottom of the survival curve indicating the number of patients surviving in each group at 20-month intervals. The dashed lines on the two curves represent censored observations. The survival analysis demonstrated in this figure is taken from the CRT registry and MACE outcomes grouped by HF aetiology is the comparison being made. IHD = Ischaemic Heart Disease.

3.8.6.2 Cox Proportional Hazard Regression

Cox proportional hazards regression is an important model for the analysis of survival data of multiple variables simultaneously. This regression model is semiparametric, making fewer

assumptions than parametric but more than non-parametric models. Most importantly it makes assumption about the shape of the baseline hazard function, unlike other parametric regression models.[284] A non-linear relationship is assumed between the hazard function and the predictors in the model. The proportional hazards model assumes the ratio is approximately constant over time where the predictor variables remain constant in the same period. This assumption is called the proportional hazards assumption.[284] This allows calculation of the hazard ratio, which represents the potential the event will occur, per unit time. The variables are entered into the model in a stepwise fashion similar to that described in the logistic regression section. The strongest predictive model is built for the pre-selected potential predictors for cardiovascular outcomes.

3.8.7 Inter-Rater Variability Echocardiography Study

An inter-rater variability study was performed to assess the degree of homogeneity between the two observers. Prof Alan Nevill (University of Wolverhampton) provided support in designing the inter-rater statistical methods and analysis.

To assess inter-rater variability between available comparative LV volume measurements several statistical analyses were performed. Firstly inter-observer correlation was performed with persons or spearman ranks correlation analysis and a correlation coefficient (r) calculated as discussed earlier in this chapter. Correlation analysis only allows the strength of a linear association to be assessed and does not account for agreement between

observers.[281] Inter-rater variability of LV volumetric measurements between observers was assessed using the concordance correlation coefficient.[285] Paired measurement assessment was undertaken with paired T-tests. Inter-observer agreement was assessed with a Bland-Altman plot.[281,286] The Bland-Altman plot quantifies agreement between two quantitative variables by building limits of agreement.[281,286] These limitations are calculated by using the mean and standard deviations of the differences between the two measurements. A scatter graph is plotted to assess the assumptions of normality of the differences.[281,286] The difference between the variables is plotted against the mean difference.[281,286] The Bland-Altman plot does not set limits of accepted agreement, more it documents the bias and states the limits of agreement.[281,286] Statistics specifically used to assess inter-observer variation in transthoracic echocardiography have previously been described in detail.[255,256]

Chapter Four

CARDIAC EXTRACELLULAR MATRIX AND CARDIAC RESYNCHRONISATION THERAPY: A SYSTEMATIC REVIEW

4.1 INTRODUCTION

Cardiac ECM biomarkers present a potential target to identify a credible predictor of CRT response. Adverse LV remodelling underpins HFrEF development, progression and severity. Cardiac ECM remodelling underpins the development and progression in adverse LV remodelling. Turnover of ECM alters in HF and with reverse cardiac remodelling following CRT implantation, this may offer potential biomarkers for CRT response prediction.[137] A systematic review was undertaken to examine the current evidence on the value of ECM biomarkers in predicting CRT response.

4.2 METHODOLOGY

Our systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-analysis [PRISMA] guidelines.[287] It was prospectively registered with PROSPERO (CRD42016025864), an international registry of systematic reviews. A protocol was designed and implemented prospectively in-line with PRISMA-P 2015[288] (**Appendix A**).

4.2.1 Eligibility Criteria

Strict eligibility criteria were applied to minimise heterogeneity of included articles. Observational studies (prospective or retrospective) and RCTs (including sub-studies) were included; basic science and review articles were excluded. Included study population's represented HF patients meeting international CRT implant guidelines.[47] Studies had to be conducted on adults (age $\geq$18years). Articles were included if they examined an ECM biomarker previously reported to predict HF outcomes, summarised in **Table 4.1**.[94] Baseline

ECM biomarkers, measured when patients were clinically stable prior to implantation, had to be compared to a pre-defined CRT 'response' criteria to evaluate their predictive value. CS sampling and long-term trends in peripheral ECM biomarker behaviour were analysed if present.

Table 4.1 Extracellular Cardiac Matrix Candidate Biomarkers in Heart Failure (Adapted[94])

Extracellular Cardiac Matrix Remodelling Biomarkers	Specific Biomarkers
Matrix Metalloproteases	MMP-1, MMP-2, MMP-9
Tissue Inhibitors of Metalloproeinase-1	TIMP-1
Collagen Propeptides	PINP, PICP, PIIINP
Collagen Telopeptides	CITP or ICTP
Myostatin	
Syndecan-4	
Galectin-3	

A variety of clinical, functional or echocardiographic criteria and cardiovascular outcomes have been used to define CRT response in studies,[1] which often correlate poorly. All response criteria were included in the review. Cardiovascular outcomes could form part of a response definition or be presented separately, their absence was not an exclusion criterion.

4.2.2 Database Search Strategies

Detailed searches were conducted on PubMed, Ovid SP MEDLINE, Cochrane Library (CENTRAL) and TRIP in February 2016 by one author and reviewed by another

independently. The search strategy used specific terms (*cardiac resynchronization therapy/cardiac pacing/extracellular matrix)* in combination, within titles/ abstracts or Medical Subject Headings. Specific circulating biomarkers (*'TIMP' 'MMP' 'collagen' 'Myostatin' 'Syndecan-4' and 'Galectin-3')* were included in the search. **Appendix B** shows the complete PubMed search. A grey literature search involved searching the international Clinical Trials database (www.clinicaltrials.gov) and international cardiology conferences (European Society of Cardiology, American Heart Association, American College of Cardiology) indexes for ongoing, abstracts and unpublished work. All included articles had their references searched for relevant publications. A date limitation of the last 15 years (31/12/1999 – 31/12/2015) was applied. No language restrictions were applied.

Title and abstract reviews were performed independently, consensus on eligibility criteria was required to be taken forward to full paper review; any conflicts were decided by an independent reviewer. Duplications of articles or cohort use were identified and only the most relevant (decided by consensus) taken forward. The Critical Appraisal Skills Programme checklist (dependent on study design) was applied to full paper review to guide evaluation of article quality.[289] Consensus had to be reached on full paper reviews before being selected for inclusion; where consensus was not reached a third reviewer made the final decision. Contact was attempted with all included article authors and any others at full paper review that were indicated.

4.2.3 Data Extraction and Management

Full texts of included articles were obtained. Pilot data extraction was performed on two randomly selected articles and reviewed for robustness. A standardised data extraction form was created to collect data on each study's design (eligibility criteria, methodology, assessment period), patient population (numbers, age, gender, aetiology, ECG, LV geometry, QoL, NYHA, functional assessment), circulating biomarker/predictor (specific ECM surrogate biomarkers, units, conditions of sampling, laboratory assessment, statistical prediction model) and outcome (response definition and cardiovascular outcomes). Data extraction was performed by two independent reviewers, a third independent reviewer resolved any disagreement.

4.2.4 Risk of Bias Assessment

Risk of bias for each study was assessed by two independent qualified reviewers utilising either the Risk of Bias Assessment Tool for Nonrandomised Studies or the Cochrane Collaboration 'Risk of Bias' assessment tool.[290,291] Both have established criteria to examine selection bias, exposure measurement, blinding and completeness of outcome data.[290,291]

4.2.5 Data Synthesis and Analysis

A descriptive synthesis was performed to summarise findings of all selected articles. A meta-analysis of included study data for each specific ECM biomarker was not achievable due to wide heterogeneity in study designs and different response definitions. Evaluation of study designs, defined outcomes and cohort characteristics were performed. The same biomarkers compared in different included articles were compared. Continuous variables

were summarised using the same units for each variable in the original text. Data was presented as mean ± standard deviation (SD), unless specified otherwise.

4.3 RESULTS

Figure 2.1 shows the screening and selection of published articles. Six articles met the inclusion criteria. Two abstracts,[292,293] and one clinical trial entry (www.clinicaltrials.gov) [NCT15019908] were taken to full review (for potential inclusion). Related articles and information were sought, including contacting authors (all 3 kindly responded). None yet had articles published and additional information provided led to exclusion from review (no baseline biomarkers taken[292] or the study design did not test biomarkers as predictors[293]).

4.3.1 Study Design

Five prospective cohort studies and 1 RCT sub-study[11] were included. **Table 4.2** summaries the different study designs and CRT response outcome definitions used. Studies selected were published between 2008 and 2014. Risk of bias was assessed in each study using the appropriate quality check tool (**Table 4.3a and 4.3b)**. The lowest risk of bias was in the single RCT sub-study.[11] The prospective cohort studies varied minimally in their bias assessment and none were excluded.

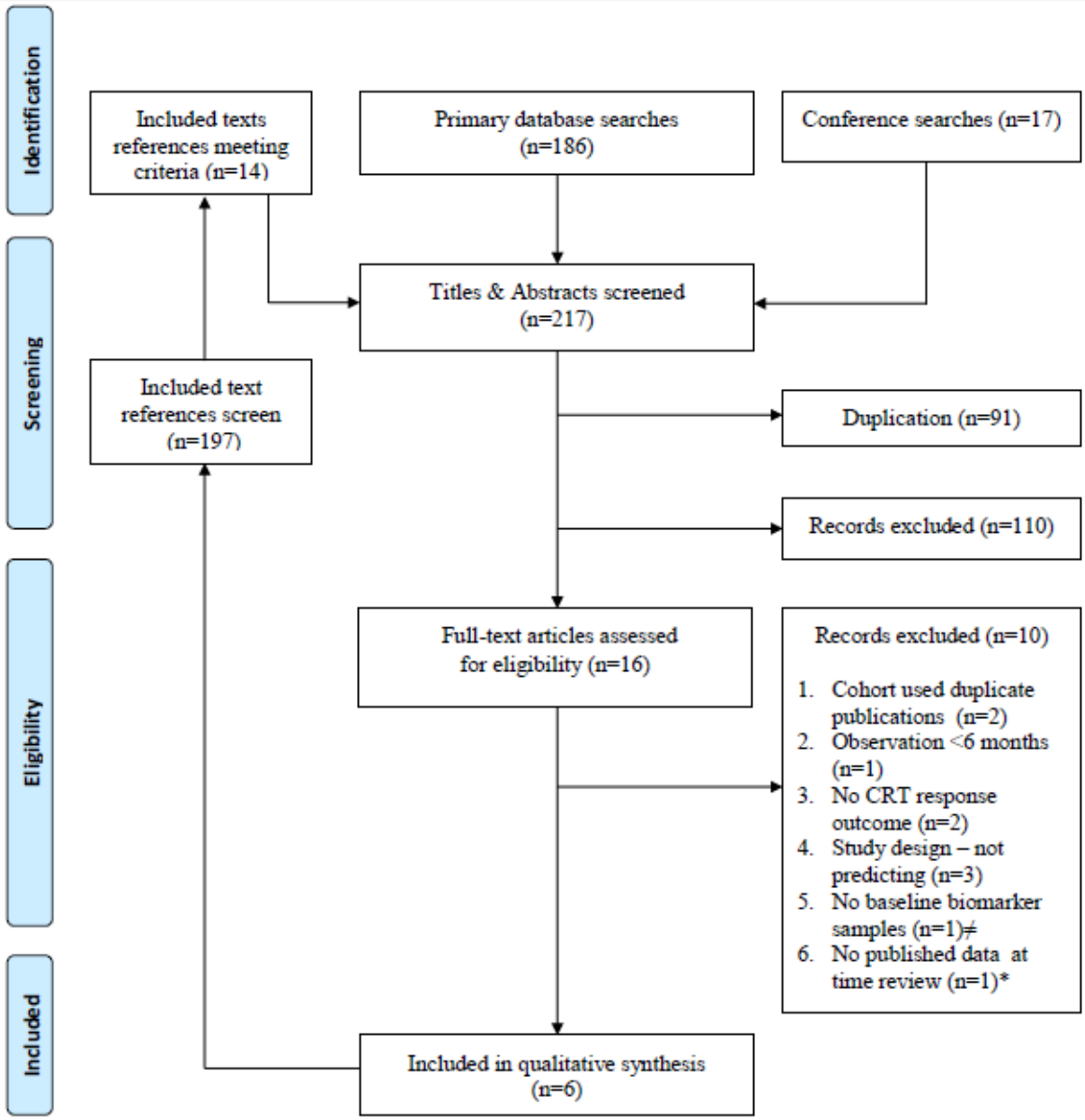


Figure 4.1 Screening and selection of articles for systematic review (Adapted[287]) ≠ Author contacted, poster presentation sent and no baseline ECM biomarker sample taken[169292]* Clinical trial [NCT15019908] author contacted and manuscript in preparation

Garcia-Bolao *et al*[126] stated 61 participants were consented; during the observation period there were 4 mortalities (3 cardiac/1 non-cardiac) and 1 functional assessment not performed at follow-up (6-minute walk test not completed due to stroke). The cohort was

59 but no explicit statement about the two exclusions made. Lopez-Andres *et al*[11] published a sub-study in 2012 of the CARE-HF[1] RCT which itself was published in 2005, interpretation of results is within this context. All studies included NYHA III-IV patients (mostly NYHA III). Two studies recruited NYHA II patients[294,295] with one also requiring a bradycardia pacing indication.[294] All studies included QRS duration≥120msec, except Garcia-Bolao *et al,*[126] who had a QRS≥130msec. In the CARE-HF trial those with a QRS duration 120-149msec needed cardiac dyssynchrony on echocardiography.[23,125] All transvenous LV leads were implanted preferably to the most lateral position possible. Dong *et al,*[244] performed only de-novo CRT-defibrillator (CRT-d) implants. Three studies[119,244,294] commented on RV lead placement with two[244] explicitly aiming for the RV apex. In CARE-HF (and sub-study) all had CRT-pacemaker (CRT-p) devices only.[23,11] CRT response definitions varied between included studies. Broadly, response definitions used were classified as 3 clinical and 3 echocardiographic. Reported response rates varied between 48.9% and 71.8% (**table 4.2**).

Table 4.2 Summary of included articles study designs and response definitions

Study ID	Design	Participants Recruited	HF/CRT Participants	Inclusion Criteria	Observation Period	Assessment's	ECM Biomarkers	CRT Responder Definition	Response Rate
Dong 2011[244]	Prospective observational	65 (20 healthy controls)	45	LVEF≤35%, NYHA III-IV, QRS ≥120msec, SR & OMT	6 months	Baseline / 3 months / 6 months: NYHA, 6MWT, TTE, Blood samples	PIIINP	↓≥15% LVESVi & Survived at 6 months	22(48.9%)
Tolosana 2010[294]	Prospective observational	55 (13 excluded after recruitment)	42	LVEF≤35%, NYHA III-IV, QRS ≥120msec & OMT or cardiac pacing indication (LVEF ≤35%)	12 months	Baseline / 6 months / 12 months: NYHA, QoL (MLHFQ), 6MWT, TTE, ECG, Blood samples	MMP-2 TIMP-1	↑≥ 10% 6MWT or if test not performed ↑> 1 NYHA & Survived/No heart transplant at 12 months	25(59.6%)
Truong 2014[295]	Prospective observational	73	73	LVEF ≤35%, NYHA II-IV, QRS≥120msec, OMT, HF decompensation ≤12 months	24 months (IQR 20.4-24.0)	Baseline: NYHA, ECG, TTE 1 month / 3 months 6 months Clinical FU	Gal-3	Improvement HF clinical composite score[93] at 6 months	40(54.7%)
Umar 2008[119]	Prospective observational	64	64	LVEF ≤35%, NYHA III-IV, QRS ≥120msec	6 months	Baseline / 6 months: NYHA, TTE; QoL (MLHFQ), 6MWT, Blood samples	PINP PIIINP ICTP proMMP-1 TIMP-1	↓≥10% LVESV at 6 months	46(71.8%)
Garcia-Bolao 2008[126]	Prospective observational	61	59	LVEF≤35%, NYHA III-IV, LBBB, QRS≥130msec, OMT	12 months	Baseline / 12 months: NYHA, QoL (MLHFQ), 6MWT, TTE, ECG, Blood samples	PICP CITP MMP-1 MMP-2 MMP-9 TIMP-1	↑≥10% 6MWT & Survival from cardiac mortality at 12 months	35(59.3%)
Lopez-Andres 2012[125]	Sub-Study Randomised Control Trial: CARE-HF	260 (CARE-HF Cohort available)	132 (CRT-P only)	LVEF ≤35%, NYHA III-IV, QRS ≥150msec or 120-149msec with echocardiographic dyssynchrony, OMT	Sub-study: 18 months CARE-HF: 29.4 months (range, 18.0-44.7)	Sub-study - Baseline / 3 months / 18 months: TTE, Blood samples	PINP PIIINP ICTP MMP-1 Gal-3	Survival & LVEF more than 35% at 18 months	CRTp(n=108): 72 (66.6%) OMT(n=117): 103(88.0%) *p*=0.0001

Table 4.3 Risk of Bias Tables

Table 4.3a Risk of Bias Assessment Tool for Nonrandomised Studied (Adapted[291])

Study	Selection of Participants	Confounding Variables	Measurement of Exposure	Blinding of Outcome	Incomplete outcome data	Selective Reporting
Dong 2011[244]	Low	Low	Low	Low	High	High
Tolsana 2010[294]	Low	Low	Low	Low	Low	High
Truong 2014[295]	High	Low	Low	Low	Unclear	High
Umar 2008[119]	Low	Low	Low	High	High	High
Garcia-Bolao 2008[126]	Low	Low	Low	High	High	High

Table 4.3b Cochrane Collaboration 'Risk of Bias' assessment tool (Adapted[290])

Study	Sequence Generation	Allocation Concealment	Blinding of Personnel	Blinding of outcome	Incomplete outcome data	Selective reporting	Other threats of validity
Lopez-Andres 2012[125]	Low	Low	High	Low	Low	Unclear	Low

4.3.2 Baseline Characteristics

A total of 415 patients were included in the systematic review. **Table 4.4** summarises the baseline characteristics of each included article. The five prospective observational studies had mean age of 67±10 years[119,126,244,294,295] (Lopez-Andres *et al*[125] excluded as presented as median and interquartile range [IQR]). Pooled here were 315 (75.9%) males in included studies, ranging between 67.8%[126] and 83.6%[295]. There was a large variation in frequency of CRT-d /CRT-p implants in each study with two not providing this data.[119,295] One study included a high proportion of device upgrades;[295] the CARE-HF trial excluded upgrades,[23,125] the remaining four studies did not state upgrades status.[119,126,244,294] AF was included in three prospective observational studies;[126,294,295] one did not report on AF or related publications.[119 120] Precise QRS duration was not stated in two studies.[244,294] Reporting of LV volumetric data varied between included studies. Three reported unadjusted LV end-systolic volume (LVESV) and LV end diastolic volume (LVEDV) data which were similar to each other (**table 4.4**).[119,294,295] Dong *et al* [244] presented LVESV and LVEDV volume indexed figures only. Garcia-Bolao *et al*[126] provided LVEF only. LVEF was compared between the five prospective cohorts and showed similar mean LVEF between 25–27%.[119,126,244,294,295]

4.3.3 Responder vs Non-Responders

Response status (Responders vs. Non-responders [RvsNR]) was presented in four of the included studies.[119,126,244,294] Truong *et al*[295] did not provide characteristics of those defined by response. Lopez-Andres *et al*[125] outlined characteristics by allocation to CRT-p vs. Optimal Medical Therapy (OMT), however not by response. There were some baseline characteristic differences between the four studies for RvsNR;[119,126,244,294] Dong *et al*[244] demonstrated

differences between RvsNR for LBBB status (15 [68.3%] vs 9 [39.1%], *p*=0.05) and ischaemic aetiology (9 [40.9%] vs 17 [73.9%], *p*=0.03). Tolosana *et al*,[294] reported lower creatinine levels in RvsNR (1.25±0.3mg/dl vs 1.76±0.8mg/dl, *p*=0.01). Umar *et al*,[119] reported responders were older and had longer QRS duration than non-responders (age: 66±10yrs vs 60±11yrs, *p*=0.03; mean±standard error QRS: 165±3msec vs. 135±8msec, *p*=0.001). Notably Hessel *et al,* published a study using the same cohort as Umar *et al,* and reported no difference in QRS duration for RvsNR [165±2msec vs. 153±3msec, *p*=NS], suggesting one of these studies has recorded it incorrectly.[119,120]

4.3.4 Extracellular Matrix Biomarkers in the Heart Analysis

A total of 9 individual ECM biomarkers were examined across all the included studies. **Table 4.5** outlines the ECM assessments and the specific laboratory techniques employed within the six included studies. Several biomarkers across studies utilise different blood products or laboratory techniques for their analysis. PIIINP utilises radioimmunoassay for all assessing studies.[119,125,244] MMP-2 and TIMP-1 utilise ELISA from different companies.[119,294]

4.3.4.1 Extracellular Matrix Biomarkers

All ECM biomarker baseline concentrations and magnitude of association (if tested) are summarised in **Table 4.6**. CS expression and trends for each ECM biomarker expression following CRT are discussed below. Lopez-Andres *et al*[125] did not provide baseline concentrations by response status, but comparison was made with the control group. Umar *et al*[119] showed baseline results for expression of ECM biomarkers studied. However, for

PIIINP non-responders no baseline concentration was reported in the article, however no statistical significance is reported RvsNR.[119]

Table 4.4 Baseline Characteristics of included articles in Systematic Review

Study ID	Age (years)	Male Gender	CRT-D	Device Upgrade	Ischaemic Aetiology	Atrial Fibrillation	Medication	LBBB	QRS (msec)	NYHA	6MWT (M)	LV
Dong 2011[244]	68±9	37 (82.2%)	45 (100%)	Not Reported	26(57.8%)	Chronic AF excluded	ACEi/ARB 27(60.0%) BB 41(91.1%)	23 (53.3%)	≥120	3.03±0.33	351±186	LVESVi 77±26ml/m2 LVEF 26±5%
Tolosana 2010[294]	66±8	35 (83.3%)	25 (59.5%)	Not Reported	19(45.2%)	8(19%)	ACEi/ARB 33(78.5%) BB 27(64.3%) MRA 20(47.6%)	Not Reported	≥120	≥III=33(78.5%)OR II = 9 (21.4%) → Pacing indication	232±126	LVESV 162±63ml LVEDV 212±66ml LVEF 27±7%
Truong 2014[295]	68±12	61 (83.6%)	Yes	41(56.2%)	39(53.4%)	34(46.5%)	ACEI/ARB 57(78.1%) BB 64(87.7%) MRA 16(21.9%)	39 (53.4%)	168±27	2.9±0.4	Not done	LVESV 163±60ml LVEDV 226±73ml LVEF 27±7%
Umar 2008[119]	64±11	52 (81%)	Yes	Not Reported	45(70.3%)	Not Reported	Not Reported	Not Reported	162±24	3.1±0.2	330±114	LVESV 172±69ml LVEDV 229±78ml LVEF 25±8%
Garcia-Bolao 2008[126]	69±4	40 (67.8%)	33 (55.9%)	Not Reported	30(50.8%)	11 (18.6%)	ACEI/ARB 59(100%) BB 34(57.6%) MRA 21(35.6%)	51 (86.4%)	158±35	3.1±0.6	327±112	LVEF 25±5%
Lopez-Andres 2012[125]*	66 (59-71)	90 (68%)	0(0%)	Excluded in CARE-HF	53(40.2%)	AF excluded	ACEi/ARB 131(99.2%) BB 88(66.7%) MRA 73(55.3%)	Not Reported	160 (152-180)	3.0±0.2	Not done	(n= 115) LVESV 206ml (174-272) LVEDV 274ml (233–355) LVEF 25% (21-29)

Table 2.5 Extracellular cardiac matrix biomarker analysis summary

Study ID	ECM Biomarkers	Other Biomarkers	Blood Material Extracted	Storage (°C)	ECM Biomarker Laboratory Analysis	Laboratory Analysis Blinding	CS Sampling	Post-Implant Sampling
Dong 2011[244]	PIIINP	NT-pro-BNP Nerve Growth Factor, Norepinephrine	Plasma	-80	PIIINP: Radioimmunoassay	Not Reported	Yes	Yes
Tolosana 2010[294]	MMP-2, TIMP-1		Serum & Plasma	-80	MMP-2: Sandwich ELISA (Serum) TIMP-1: Sandwich ELISA (Plasma)	Not Reported	Yes	No
Truong 2014[295]	Gal-3	NT-*pro*-BNP	Plasma	-80	Gal-3: ELISA sST2: ELISA	Yes -blinded source and type of sample	Yes	No
Umar 2008[119]	PINP PIIINP ICTP proMMP-1 TIMP-1	NT-*pro*-BNP	Serum & Plasma	-80	PINP: Radioimmunoassay (Serum) PIIINP: Radioimmunoassay (Serum) ICTP: ELISA (Serum) ProMMP1:ELISA (Serum) TIMP-1: ELISA (Plasma)	Not Reported	No	Yes
Garcia-Bolao 2008[126]	PICP CITP MMP-1 MMP-2 MMP-9 TIMP-1		Serum	-40	PICP: ELISA CITP: Radioimmunoassay MMP-1: ELISA MMP-2: sandwich ELISA MMP-9: sandwich ELISA TIMP-1: ELISA	Not Reported	No	Yes
Lopez-Andres 2012[125]	PINP PIIINP ICTP MMP-1 Gal-3	NT-*pro*-BNP	Serum	-80	PINP: Radioimmunoassay PIIINP: Radioimmunoassay ICTP: Radioimmunoassay MMP-1:Radioimmunoassay Gal-3: Radioimmunoassay	Yes, central laboratory	No	Yes

Table 4.6 Baseline extracellular cardiac matrix associations with CRT response

ECM	Study ID	Baseline	Model	Predicting Response
PINP	Umar 2008[119]~	**TC: 35.4±5.0ug/l**, R: 32.9±2.2ug/l NR: 41.9±4.3ug/l, *p*=0.04	Multiple Logistic Regression $	Univariate: OR 0.99, CI(95%) 0.93–1.00, *p*=0.05 Multivariable: OR 0.96, CI(95%) 0.93-0.99, *p*=0.03
	Lopez-Andres 2012[125]*	**CRT-p: 33.0ug/l(24.6–49.4) OMT: 33.1ug/l (23.0–49.3) *p*=NS**	Multiple Logistic Regression Π	No association with response
PICP	Garcia-Bolao 2008[126]	**TC: 74.3±29.9ug/l,** R:85.6±29.4ug/l NR: 57.8±22.2ug/L, *p*=<0.001	ROC: PICP:CITP	AUC 0.71 CI (95%) 0.57–0.85 Cut-off 14.4 CI (95%) 9.8–17.7 Sensitivity 63% (51–80) Specificity 70% (50–85) OR 2.07 CI (95%) 0.98–4.39
PIIINP	Dong 2011[244]≠	**TC: 0.88±0.21ug/l,** R: 0.80±0.20ug/l NR: 0.96±0.19ug/l, *p*=0.03	Multiple Logistic Regression $	Univariate: OR 0.77, CI(95%) 0.62-0.97, *p*=0.03 Multivariable: OR 0.20, CI(95%) 0.03-1.17, *p*=0.07
	Umar 2008[119]~	R: 4.59±0.24ug/L NR: < responders, *p*=NS	Multiple Logistic Regression $	Univariate: OR 1.23, CI(95%) 0.86–1.76, *p*=0.23 Multivariable: OR 1.35, CI(95%) 0.94-1.93, *p*=0.1
	Lopez-Andres 2012[125]*	**CRT-p: 4.6ug/L (3.8–6.8) OMT: 4.7ug/l (3.8–6.5), *p*=NS**	Multiple Logistic Regression Π	No association with response
ICTP	Umar 2008[119]~	**TC: 3.1±0.8ug/l,** R: 3.5±0.6ug/l NR: 2.1±0.4ug/L, *p*=ns	Multiple Logistic Regression $	Univariate: OR 1.24, CI(95%) 0.93–1.66, *p*=0.13 Multivariable: No association with response
	Lopez-Andres 2012[125]*	**CRT-p: 4.1ug/l (2.6–6.0) OMT: 3.4ug/l (2.7–5.0), *p*=NS**	Multiple Logistic Regression Π	No association with response
CITP	Garcia-Bolao 2008[126]	**TC: 5.1±2.5ug/l,** R: 4.90±2.5ug/l NR: 5.3±2.5ug/l, *p*=0.51	ROC: PICP:CITP	AUC 0.71 CI (95%) 0.57–0.85 Cut-off value 14.4 (CI 95% 9.8–17.7) Sensitivity 63% (51–80) Specificity 70% (50–85) OR 2.07 CI (95%) 0.98–4.39)
Pro-MMP-1	Umar 2008[119]~	**TC: 7.7±0.8ug/l,** R: 7.6±0.7ug/l NR: 8.0±1.1ug/L, *p*=0.71	Multiple Logistic Regression $	Univariate: OR 0.97, CI(95%) 0.87–1.09, *p*=0.71 Multivariable: No association demonstrated
MMP-1	Garcia-Bolao 2008[126]	**TC: 8.9±11.4ug/l** R: 7.3±10.5ug/l NR: 11.3±12.5ug/l, *p*= 0.17	ROC	Not performed as no difference Baseline MMP-1:TIMP-1 Ratio: 0.005±0.001 R: 0.004±0.0007 vs NR: 0.0063±0.0008, *p*=0.297
	Lopez-Andres 2012[125]*	**CRT-p: 2.7 ug/l (2.1–3.5) OMT: 2.7ug/l (2.0–3.9), *p*=NS**	Multiple Logistic Regression Π	Univariate ≤3ug/l: OR 2.42, CI(95%) 1.23–4.76 *p*=0.011, Multivariable ≤3ug/l: OR 3.04 CI(95%) 1.37–6.71, *p*<0.01
MMP-2	Tolosana 2010[294]	**TC:295±70ug/l,** R:258±56ug/l NR:325±116ug/l, *p*=0.02	Cox Regression Model $	Univariate: difference already noted (p=0.02) Multivariable: No association demonstrated
	Garcia-Bolao 2008[126]	**TC: 1434±401.5ug/l** R: 1393.8±374.5ug/l NR: 1496.6±438.9ug/l, *p*=0.36	ROC	Not performed as no difference demonstrated
MMP-9	Garcia-Bolao 2008[126]	**TC: 44.7±23.2ug/l,** R: 41.1±22.8ug/l NR: 49.9±23.3ug/l, *p*=0.17	ROC	Not performed as no difference demonstrated
TIMP-1	Tolosana 2010[294]	**TC:242±61ug/l**, R:216±50ug/l NR:277±59ug/l, *p*=0.001	Cox Regression $; ROC	Multivariate: OR 0.97, CI(95%) 0.96–0.99, *p*=0.005 ROC: ≥248 ug/l, Sensitivity 71%, Specificity 72%, OR 6.8 CI (95%) 1.5–31)
	Umar 2008[119]~	**TC: 120.3±8.2ug/l,** R: 124±5.2ug/l NR: 111±7.1ug/l, *p*=0.16	Multiple Logistic Regression $	Univariate: OR 1.01, CI(95%) 0.99-1.03, *p*=0.16 Multivariable: No association demonstrated
	Garcia-Bolao 2008[126]	**TC: 488.9±249.5ug/l** R: 437.5±136.5ug/l NR: 563.8±345.7ug/l, *p*=0.135	ROC	Not performed as no difference Baseline MMP-1:TIMP-1: TC: 0.005±0.001 R: 0.004±0.0007 vs NR: 0.0063±0.0008, *p*=0.297
Gal-3	Truong 2014[295]*	**TC: 18.1ug/l (14.0-23.0)** Positive result ≥25.9ug/l	2x2 Table; McNemar test	Peripheral: Sensitivity 15% (5–32), Specificity 80% (64-91), PPV: 38% (14–68), NPV: 53% (40–66)
	Lopez-2012[125]*	**CRTp: 25.7ug/l(20.6-31.4) OMT: 25.1ug/l(19.6–30.9, *p*=NS**	Multiple Logistic Regression π	No association with response

NPV=Negative predictive value, NR=Non-responder, NS=Not significant, PPV=Positive predictive value, R=Responder, ROC=Receiver operator curve, TC=Total cohort, ≠biomarker mean±SD given in logarithmic format, ~ mean±SE margin, * median (IQR) given, data represented as CRT-p & OMT groups, Π=Statistical model predicts 'non-response', $=Statistical model predicts 'response'

2.3.4.2. PINP/PICP

PINP and PICP share a 1:1 stoichiometric relationship with the collagen molecule, therefore, they were considered together. Umar *et al*[119] reported similar total cohort means values to Lopez-Andres *et al*[125] median values (the skew of this data is unknown). Umar *et al*[119] observed higher PINP baseline level predicted poor response. Garcia-Balao *et al*[126] reported the opposite for PICP. Lopez-Andres *et al,*[125] observed no significant association of baseline PINP with CRT response or other outcomes. Variation in pattern of reported levels between the 3 studies were likely due to differences in response definitions and baseline characteristics. Garcia-Balao *et al*[126] utilised a clinical definition of response, whereas the other two studies used echocardiographic criteria.[119,125] All studies varied in duration of follow-up. Umar *et al*[119] had a higher proportion of men with ischaemic aetiology than the other studies. Lopez-Andres *et al,*[125] excluded AF, whereas within the Garcia-Balao *et al*[126] cohort it was present in 18.6% of participants. Garcia-Balao *et al*[126] tested the predictive value of type I collagen turnover with the PICP:CITP ratio with a ratio $\geq$14.4 predicting response.

Variation was reported in type I collagen synthesis following CRT. Lopez-Andres *et al,*[125] observed no difference in PINP levels between CRT-p and OMT in the short and long-term; there was no analysis of RvsNR.[125] Umar *et al,*[119] observed responders had increased PINP levels at six months compared to baseline (32.9±2.2 ug/l vs 46.7±4.0 ug/L, p=0.001) with no differences in non-responders.[119] In contrast, Garcia-Balao *et al*,[126] demonstrated from baseline to one year follow-up PICP levels decreased in responders (85.6+29.4ug/l vs

71.5+24.1ug/l, p< 0.001) and increased in non-responders (56.6±21.7ug/l vs 88.7+43.5ug/l p< 0.001).

2.**3.4.3. PIIINP**

Variation was reported in trends of PIIINP levels at baseline. Dong *et al*,[244] reported logarithmic figures making absolute figure comparison challenging. Geometric means could be calculated, but given the small numbers of participants this was likely to underestimate the true mean.[244] Higher PIIINP levels in HF vs healthy controls (0.88±0.21ug/l vs 0.71±0.14ug/l, *p*=0.01) were observed.[244] Responders had significantly lower PIIINP baseline levels than non-responders (*p*=0.03).[244] Umar *et al*[119] demonstrated no difference in baseline levels between RvsNR. Lopez-Andres *et al*[125] reported similar baseline levels between CRT-p vs OMT, but did observe PIIINP (>4.7ug/l) in univariate analysis predicted cardiovascular outcomes (death or HF hospitalisation at 18 months) (OR 1.80, 95% CI 1.06–3.06, *p*=0.03).[125]

CS and peripheral arterial expression of PIIINP was similar prior to CRT, in a sub-set of the Dong *et al*[244] cohort (n=36/45). There was no difference in PIIINP expression for the entire cohort comparing baseline with follow-up (0.88±0.21ug/l vs 0.87±0.20ug/l *p*=0.22); no results were provided for RvsNR.[244] Lopez-Andres *et al*,[125] observed no difference between CRT-p and OMT groups from baseline to short and long-term follow-up. In contrast, Umar *et al*,[119] observed responders had increased PIIINP expression between baseline and six months (4.59±0.24ug/l vs 5.13±0.36 ug/l, *p*<0.05) but no difference in non-responders.

4.3.4.4 ICTP or CITP

Both ICTP and CITP were used to represent carboxyl-terminal peptides of type I collagen in 3 included studies. Umar *et al,*[13] and Garcia-Balao *et al,*[12] demonstrated similar baseline means for ICTP/CITP for the entire cohort. Neither was identified as independent predictors of CRT response.[119,126] Garcia-Bolao *et al,*[126] identified that PICP:CITP ratio strongly predicted response but was driven by PICP. Lopez-Andres *et al*,[125] observed similar expression between CRT-p and OMT groups and showed no predictive value. Lopez-Andres *et al*[125] observed that ICTP behaviour following CRT-p implant demonstrated no significant change at short and long-term follow-up. Umar *et al*[119] supported this observation as no significant changes were observed at six months. However, Garcia-Bolao *et al,*[126] observed that there was a trend towards lower CITP levels at one year follow-up for responders (4.9±2.5ug/l vs 2.6±2.7ug/l, *p*=0.122) but no change in non-responders.

4.3.4.5 MMP-1, MMP-2, MMP-9

There were variations in reported baseline concentrations for MMP-1. The mean for MMP-1 in Garcia-Bolao *et al,*[126] was higher than median observed in CRT-p and OMT groups in Lopez-Andres *et al,*[125] though the data skew is unknown. Garcia-Bolao *et al,*[126] examined the predictive value of MMP-1:TIMP-1 given their intrinsic regulatory role in collagen turnover,[113] but showed no statistical significance. Lopez-Andres *et al,*[125] observed with a baseline MMP-1 $\leq$3ug/l an adjusted 3-fold increased risk of CRT non-response and an increased risk of death or NT-*pro*-BNP >1000ng/l (OR 2.23, 95% CI 1.00–5.00, *p*=0.051/0.073 adjusted with/without renal function).[125] A precursor to MMP-1 called pro-matrixmetalloproteinase-1 (pro-MMP-1) was studied by Umar *et al.*[119] They observed no difference in baseline pro-MMP-1 expression between RvsNR.[119]

Two studies reported cohort means for MMP-2 baseline concentration with large differences (**Table 4.6**). Responders had lower MMP-2 baseline concentrations in both studies. Tolosana *et al,*[294] reported a significant difference between RvsNR (*p*=0.02), whereas Garcia-Bolao *et al,*[126] demonstrated no difference. The differences are not fully explained by study design, response definition or cohort characteristics as they showed similarities (**Tables 4.2** and **4.4**). Variation in levels may be due to Tolosana *et al,*[294] using plasma and Garcia-Bolao *et al,*[126] using serum in their sandwich ELISA's (**Table 4.5**). MMP-9 was reported by Garcia-Bolao *et al*[126]*,* who observed a trend towards lower baseline MMP-9 concentration for responders. Baseline MMP-9 did not predict CRT response.[126]

Lopez-Andres *et al,*[125] observed no change in concentration of MMP-1 from baseline to short and long term follow-up for both CRT-p and OMT groups. Garcia-Bolao *et al*,[126] observed MMP-1 levels increased significantly for responders at 1year follow-up (7.33±10.5 ug/l vs 10.68±10.5ug/l, *p*= 0.032) compared to no significant difference in non-responders (*p*=0.47). A precursor to MMP-1 called pro-matrixmetalloproteinase-1 (pro-MMP-1) was studied by Umar *et al,*[119] and found not to change significantly from baseline to follow-up at six months.[119]

Garcia-Bolao *et al*[126] observed MMP-2 expression did not alter at one year follow-up for both responders and non-responders.[126] Tolosana *et al*[294] observed MMP-2 had lower expression in CS than peripheral samples for responders (239±78ug/l vs 258±56ug/l) and non-responders (312±70ug/l vs 325±116ug/l), however this was not statistically compared. The mean of baseline MMP-2 expression for both participants who reverse LV remodelled

(↓≥10% LVESV at 12 months) and those that did not demonstrated no significant difference in CS (230±55ug/l vs 307±100ug/l, *p*=0.08) or peripherally (267±63ug/l vs 299± 100, *p*=0.32).[294] CS MMP-2 did not predict CRT response.[294] During their one year follow-up, Garcia-Bolao *et al*[126] observed MMP-9 levels increased significantly (41.1±22.8ug/l vs 50.9±25.2ug/l, *p*=0.032) yet non-responders showed no change at a year (50.7±23.3 vs 50.9±21.2, *p*=1.0).[126]

4.3.4.6 TIMP-1

Tolosana *et al*[294] observed responders had significantly lower concentrations at baseline of TIMP-1 than non-responders. Neither Umar *et al*[119] nor Garcia-Bolao *et al*[126] observed a significant difference in baseline TIMP-1 concentration between RvsNR. Higher peripheral TIMP-1 was identified as an independent predictor of non-response by Tolosana *et al*[294] in multivariable analysis; a concentration of ≥248ug/l had a 71% sensitivity and 72% specificity for predicting non-response. However, Umar *et al*[119] did not identify TIMP-1 as a predictor. Garcia-Bolao *et al*[126] tested TIMP-1 in the MMP-1:TIMP-1 ratio and did not identify TIMP-1 as a significant predictor of RvsNR.

Tolosana *et al*[294] was the only study to examine CS vs peripheral concentrations of TIMP-1 with both correlating strongly (r^2=0.54). Baseline CS expression was lower than peripheral in responders (205±51ug/l vs 216±50ug/l) and non-responders (260±60ug/l vs 277±59ug/l), though this was not statistically tested.[294] CS expression of TIMP-1 was lower in RvsNR (*p*=0.003).[294] Tolosana *et al*[294] compared TIMP-1 baseline concentrations between those that did and did not LV reverse remodel and found significantly lower expression in the CS

(192±47ug/l vs 258±55 ug/l, *p*=0.001) and peripherally (208±46ug/l vs 267±60ug/l, *p*= 0.001). CS and peripheral expression was significantly higher in those 6 patients who had cardiovascular mortality during the observation period.[294] The trend in TIMP-1 concentration was noted to decrease at one year in responders (437.5±136.5ug/l vs 365.2+138.5 ug/l, *p*=0.002) by Garcia-Balao *et al*,[126] with no statistically significant decrease in non-responders (457.3+247.5 ug/l vs 344.8+110.7 ug/l p=0.084). The MMP-1:TIMP-1 ratio decreased from baseline to one year follow-up for responders (0.004±0.0007 vs 0.0066±0.001, *p*=0.007) with no change for non-responders.[126] In contrast, Umar *et al*[119] observed no change in concentration of TIMP-1 for CRT response at six months for RvsNR.

4.3.4.7 Galnectin-3

Lopez-Andres *et al*[125] reported higher baseline levels of Gal-3 than Truong *et al,*[295] due to different response definitions and variation in cohort characteristics. Lopez *et al*[125] used an echocardiographic definition at 18 months and Truong *et al,*[295] utilised HF clinical composite score at 6 months. Truong *et al,*[295] has higher ischaemic aetiology (53.4% vs. 40.2%) and included AF patients. Neither study reported baseline concentrations for RvsNR.[125,295] Truong *et al*[295] observed that peripheral baseline Gal-3 above a pre-set concentration (>25.9ug/l) had low sensitivity and high specificity for predicting CRT response.

CS expression of Gal-3 at baseline was 10% lower than peripheral (median and IQR, 16.7ug/l [12.5-21.0] vs 18.1ug/l [14.0-23.0], *p*<0.001).[295] The CS Gal-3 concentrations above the pre-set level had higher sensitivity (18%) and specificity (90%) for predicting CRT response than peripheral samples.[295] Lopez-Andres *et al*[125] observed Gal-3 did not predict CRT response.

No significant change in Gal-3 concentration was noted between baseline and long-term follow-up for both CRT-p and OMT groups.[125] No difference was noted between follow-up concentrations between both groups.[125] Lopez-Andres *et al*[125] demonstrated baseline Gal-3 concentration ≥30ug/l predicted death or hospitalization for worsening HF (OR 2.98, 95% CI 1.43-6.22, *p*=0.004).

4.4 DISCUSSION

The Cardiac ECM is a highly dynamic structure that is integral to myocardial structure and function which detrimentally remodels following cardiac injury leading to the altered turnover, replacing contractile tissue with collagen rich connective tissue and ultimately the development of myocardial fibrosis.[113] Myocardial fibrosis is characterised by adverse remodelling which contributes to systolic and diastolic HF.[113,296] PINP, PICP and PIIINP are released into the circulation during conversion and deposition of procollagen to collagen and are up-regulated during myocardial fibrosis and associated with adverse HF outcomes.[113,124,137,296] Mechanistically higher up-regulation of collagen deposition would challenge a CRTs ability to reverse remodel and for the patient to respond. Umar *et al*[119] supported this hypothesis observing significantly lower baseline PINP expression predicted echocardiographic response. Dong *et al*[244] did observe lower baseline PIIINP predicted echocardiographic response on univariate analysis, but not on multivariable analysis. In a recent observation study, Sokal *et al*[127] supports these observations with their published sub-study of the Triple Site Versus Standard Cardiac Resynchronization (TRUST CRT) trial[297] examining ECM biomarkers (PINP, PIIINP, MMP-2 & MMP-9) ability to predict echocardiographic CRT response (↑≥10% LVEF at 6 months). This study was not identified

through the systematic review as it was published in January 2016; outside the defined search window for the review.[127] The TRUST-CRT trial[297] was a single-centre, randomised, parallel observation study which was powered for differences in response (composite endpoint: survival free from HF or heart transplantation and ↑≥10% LVEF and ↑≥10% peak VO^2max and ↑≥10% 6MWD at 6 months) between triple site (two leads on LV) and standard (biventricular) pacing (randomised 1:1) for HFrEF patients (n=100) meeting criteria for a complex device. The TRUST CRT trial[297] identified triple site pacing improved functional response. Sokal *et al*[127] observed lower PIIINP concentration pre-implant independently predicted response (OR 3.6, 95% CI 1.2-10.2, *p*=0.017) supporting the findings of Dong *et al*[244] and Umar *et al*.[119] Furthermore, higher PIIINP pre-implant was observed to predict MACE (OR 3.6, 95% CI 1.5-9.2, *p*=0.007).[127] Lopez-Andres et al,[125] in a larger cohort demonstrated higher baseline PIIINP predicted worse cardiovascular outcomes.[125] Sokal *et al*[127] observed no difference in baseline expression between RvsNR for MMP-2 and MMP-9. This study offers further insights into the cardiac ECM remodelling in HFrEF patients undergoing CRT implantation and their potential as predictors of response. However the study has limitations that should be considered; the response definition changed between the TRUST CRT trial[297] and the subsequent sub-study had less participants (n=74) than the original study. The two interventions were not statistically accounted for in analysis.[127] In contrast, Garcia-Balao *et al,*[126] observed higher baseline expression of PICP in responders and PICP:CITP ratio (type-I-collagen turnover) of ≥14.4 had >2 fold increased chance of predicting functional response, driven by PICP. Critically, echocardiographic and clinical/functional response criteria correlate poorly,[1] so could not be contrasted. Importantly, Lopez-Andres *et al*[125] the largest study included in the review, did not observe up-regulation of collagen synthesis predicting echocardiographic non-response, which does

challenge the Umar *et al*[119] and Dong *et al*[244] observations; however, the cohort characteristics and study designs were different. The observations of collagen synthesis following CRT implantation conflict with each other. Umar *et al*[119] reported a significant increase in PINP and decrease in PIIINP expression in responders at six months. In contrast Garcia-Bolao *et al*[126] observed PICP levels decreased for responders and increased for non-responders at one year, which would be expected, but is based upon a functional response definition. In contrast to collagen synthesis, degradation biomarkers (ICTP or CITP) did not predict CRT response.[119,125,126] Furthermore, no significant change in ICTP or CITP expression was observed at follow-up across all 3 studies.[119,125,126] Alteration in collagen synthesis rate is observed to be more powerful at predicting response than collagen degradation. Different patterns of collagen synthesis biomarkers predicting response have been observed; lower expression predicted LV reverse remodelling[119,244] whereas higher rates predicted functional response.[126] The variation in these patterns is explained by the different response definitions and cohort characteristics. The study cohort for Umar *et al*[119] had a higher proportion of men and ischaemic cardiomyopathy than Garcia-Bolao *et al.*[126] The heterogeneities between these studies make drawing conclusions difficult. Lopez-Andres *et al*[125] also challenge any observations due to the size of cohort and no prediction value to collagen turnover observed. Overall collagen synthesis is observed to be important in predicting CRT response, especially LV reverse remodelling, with results replicated in two studies that lower rates predict LV reverse remodelling.[10,26]

MMP-1,-2 and -9 perform a critical role in myocardial collagen degradation and have been identified as being important prognostic markers in HF.[120,134,136] Predictive value for CRT non-response (death or LVEF ≤35% at 18months) was only demonstrated in baseline MMP-1

expression ≤3ug/l[125] supporting an observation by Jordan *et al*[134] that lower MMP-1 inferred worse HF prognosis. MMP-2 had large variations observed between the included studies,[125,294] but was not demonstrated to predict response. MMP-9 was only observed in one included study showing no predictive value,[126] however recently Dini *et al*[136] demonstrated raised levels (>238 ng/ml) predicted worse HF outcomes. MMP activity was not considered in any of these studies as a predictor but would be important to consider in the future. Current evidence suggests MMPs, especially -2 and -9, have not yet had their potential fully evaluated.

TIMP-1 regulates the endogenous proteolytic MMP system involving discordant inhibition and in chronic inflammatory states stimulating collagen synthesis and myocardial fibrosis.[113,294] Tolosana *et al,*[294] observed a significant baseline difference in RvsNR expression with lower TIMP-1 in responders. Tolosana *et al,*[294] demonstrated baseline TIMP-1 (≥248ug/l) predicted CRT non-response. Trucco *et al,*[298] in long-term follow-up of the same cohort demonstrated the same threshold independently predicted mortality at 60±34months, (sensitivity 80% and specificity 71%). Tolosana *et al*[294] also demonstrated statistically significant lower TIMP-1 is found in participants that do LV reverse remodel (LVESV reduction ≥10%). Umar *et al*[119] and Garcia-Bolao *et al*[126] observed no difference statistically at baseline. Variation between the reported literature in the magnitude of association of TIMP-1 exists, however Tolosana *et al*[294] offers a well-designed prospective observational study which is powered giving strength to the conclusions drawn.

Galectin-3 stimulates fibroblasts to release TIMPs and MMPs that regulate collagen turnover, resulting in myocardial fibrosis.[138] Elevated levels are independent predictors of

adverse outcomes in HF.[138] Evaluation of Gal-3 as a predictor of response was limited, as RvsNR was not reported in either of the 2 studies.[125,295] Truong *et al,*[295] demonstrated peripheral baseline Gal-3 ≥25.9ug/l had specificity for predicting CRT response. Lopez-Andres *et al*,[125] observed Gal-3 baseline expression ≥30ng/l had nearly 3-fold increased risk of death or hospitalization for worsening HF following CRT. Though not demonstrated to be a strong predictor the evidence suggests that Gal-3 is a good biomarker for predicting poor outcomes in HF and needs further evaluation.

The greatest challenge for research into CRT response and one that this systematic review demonstrated is lack of an accepted response definition. Differing definitions rarely correlate,[1] which our review clearly demonstrates. Echocardiographic and clinical/functional definitions correlate very poorly and should never be compared or applied in a composite definition; [1] LV reverse remodelling should be considered separately.[1,93]

Heterogeneity among included studies was widespread despite a rigorous eligibility and screening criteria. The variations in study design, cohort characteristics and response definitions made pooling data in a meta-analysis impractical. Multiple cardiac ECM biomarkers were being explored in this review to examine what research into ECM biomarkers and CRT had been done looking at their predictive strength. This offered a broad research question and there were a small number of studies eligible. Each was on small patient numbers and had differing study design and most importantly used incomparable response definitions. All these factors together meant a met-analysis was not feasible. CRT implantation techniques and indications have evolved over the last 15 years and offer another source of heterogeneity. Furthermore differences in laboratory techniques account

for some variation among biomarker results. All the included studies lacked individual power and had limited participant numbers making achieving any consensus conclusion very difficult. These limitations are particularly important to consider in future research studies.

Collagen synthesis biomarkers have shown the most potential, particularly PINP and PIIINP, but will require further study. MMP-2 and -9 have no conclusive predictive value, and need further investigation. The studies to date have been too small to offer conclusive evidence they can't predict response following CRT. Heterogeneity is the greatest challenge for research in this field and needs to be minimised in future studies, for instance focusing on one particular mechanism of injury e.g. hypertension, so the expression pattern of ECM biomarkers is similar in HF patients. Another step would be for an accepted definition of CRT response to be produced, to allow direct comparison to be undertaken.

4.5 Contribution of Authors

Dr Christopher McAloon had the original concept, designed methodology, performed literature search, article screening, data extraction, quality assessment, results analysis and drafted the manuscript. Dr Danish Ali performed article screening, data extraction and quality assessment. Mr Thomas Hamborg reviewed statistical interpretation. Professor Pritwash Banerjee, Dr Paul O'Hare and Dr Harpal Randeva reviewed methodology and critically edited manuscript. Dr Faizel Osman reviewed methodology, eligibility and critically edited manuscript.

4.6 PUBLICATIONS

This systematic review has been published as an open access article in Open Heart[299].

Chapter Five

PRE-IMPLANTATION PREDICTORS AT A UK TERTIARY CENTRE

5.1 INTRODUCTION

Non-response remains one of the largest challenges in management of patients undergoing CRT implantation. The current rate of non-response remains at 20-40% of all CRT implantations performed.[103] Several variables have been associated with predicting response. These variables are can be summarised as pre-implant, implant and post-implant. Unfortunately many of these variables are not proven to be robust in the prediction of response.

Debatably the pre-implantation variables are the most important to the patient as these may inform their decision to proceed. Multiple 'pre-implantation' variables; clinical background, resting ECG, echocardiography and procedural factors have been suggested as predictors of response.[38,39,65-68] Several studies (**Table 1.3**) have indicated that aetiology, gender, QRS duration/morphology, left atrial dimensions and LV dimensions can predict response, but these findings have not been reproducible in all study cohorts.[38,39,65-68] The most consistent predictor of response and cardiovascular outcomes is QRS duration on ECG.[39,68] A recent meta-analysis of five Medtronic randomised control trials (n=4317) found increasing QRS duration in patients having CRT vs. Controls (OMT/ICD/Back-up pacing) predicted all-cause mortality or HF hosptilisation (**Figure 1.2**).[39] Cleland *et al*,[39] identified several potential 'pre-implantation' predictors that might predict cardiovascular outcomes in a multivariable logistic regression model, but when treating the presence of a CRT as an interaction term, the only variable that remained a predictor was QRS duration.[39] The meta-analysis could not include individual particpants data from non-Medtronic randomised control trials, specifically COMPANION[22] and MADIT-CRT.[27] Broadly, both these trials[22,27] support Cleland et al,[39] that increasing baseline QRS duration infers less chance of having an

adverse cardiovascular outcome. Interestingly several smaller observational studies have not replicated these findings.[64-67] However, the limitations of these studies in terms of design and numbers of patients observed contributed to the lack of reproducibility.[39,63] A recurring theme with weaknesses in this field is the inconsistently applied definition of response and the challenge this poses in comparing variables and pooling data[1]; a challenge encountered during our conduct of a systematic review into ECM biomarkers to predict CRT response (**Chapter 4**).

International guidelines are informed by the patient cohorts from the large multi-centre randomised control trials.[17,22,23,27,47,56] The strongest pre-implant predictor of response has been demonstrated to be QRS duration and is reflected in the international guidelines,[17,47,56] yet non-response remains a persistent issue.[234]

5.2 AIMS (Chapter 2)

The aim of this chapter is to examine reported pre-implant predictors of response and cardiovascular outcomes in the literature within our heterogeneous single centre CHF population. This analysis will inform the selection of pre-implant variables that are examined in the phase II prospective observation study with the specified biomarkers.

5.3 CONCISE METHODS

5.3.1 Study design

A single-centre, retrospective study of all consecutive CRT implants at UHCW performed over five years (January 2009 to December 2013). **Figure 3.3** summarises the patient

selection and study design. All implants met national and international implantation guidelines.[17,47,234] All CRT-p and CRT-d implants were included, both *de novo* and upgrade implants to reflect all patients getting a biventricular pacing device. Pulse generator only changes were excluded. Procedures were excluded from analysis if LV lead implant failed or there was an immediate complication that prevented >90% biventricular pacing, as the CRT would be deemed to not be working correctly. Exclusions were applied if clinical response status at baseline or follow was not determinable from the records**.** Cardiovascular outcome data were collected for all patients until 31st December 2014 (minimum one year).Due to the outcome definition used, the low number of patients and the high percentage of non-response, this study was not powered. Approval was provided by our local Research, Development and Innovation department. **Chapter 3.4** summarises in detail the retrospective observational study design.

5.3.2 Data Collection

Detailed methodology applied to data collection for included patients is summarised in **Chapter 3.4**. Electronic (Clinical Results Reporting Systems, University Hospital Coventry and Warwickshire, Coventry, UK) and paper case notes were reviewed for patients meeting eligibility, including all correspondence from local referral centres. Hospital coding data was also reviewed for each participant. Two reviews of case records were performed to maximise recorded data quality and minimise bias. Baseline echocardiograms (EchoPac, GE Healthcare, Horten, Norway) and ECGs prior to CRT implant were reviewed and had LVEF, QRS duration and BBB morphology measured**.**

5.3.3 Implant Procedure

A detailed description of the CRT implant procedure and aftercare is outlined in **Chapter 3.2.**

5.3.4 Potential Pre-implant Predictors Model (Chapter 3.4)

Potential clinical predictors were considered prior to assessment and pre-selected based on previous reports. Predictors identified were age and gender,[38] device type (CRTp/CRTd) and upgrade status,[235] clinical aetiology,[38,65,68] CKD,[236-238] diabetes mellitus,[236] BBB morphology,[38,66,68] QRS duration,[38,39,68] and LV ejection fraction on echocardiography.[38,67] CKD status at implant was defined as a GFR <60ml/min/1.73m^2 using the modification of diet in renal disease equation.[237,239,240] Time from implant to determination of response was considered likely to be wide, therefore, time between implant and assessment was included as a confounding variable in the clinical predictor model. Baseline NYHA status was not included as a predictor due to the direct association with clinical response.

5.3.5 Outcomes: Clinical Response and Cardiovascular Outcomes (Chapter 3.4)

Primary outcome was the overall clinical response assessed at the latest cardiology/HF consultation. The criterion for clinical response was a decrease in NYHA classification $\geq$1 symptoms from baseline. The latest consultation was defined as the closest to the final observation date (31st December 2014). Secondary outcomes were acute ($\leq$12weeks), long-term (>12weeks) clinical response and MACE (all-cause mortality or first HF admission). Mortality rates were generated from hospital coding data; HF admissions were defined as a hospital admission requiring intravenous diuretics.

Determination of NYHA class was performed by reviewing all recorded consultations. The latest NYHA classification available was used to determine response. Clear documentation of NYHA score by the clinician was used to determine symptom classification. Where no clear scoring was made, the content of the recorded consultation was reviewed by three clinicians (blinded to each others review) to determine NYHA class. Where consensus was not reached the patient was excluded from final analysis.

5.3.6 Statistical Analysis (Chapter 3.8)

Categorical variables were reported as frequency and percentage. Comparison analysis for categorical data was performed using Chi-Squared and where required Fisher's Exact test. Continuous data underwent histogram plots for assessment of normality. Normally distributed data were reported as mean±SD and comparative analysis performed using independent t-tests. Non-normally distributed data were reported as median (range) and compared using a Mann-Whitney U test. Univariate logistic regression analysis was performed on all pre-identified variables; those achieving p-value <0.15 were pooled as covariates for multiple logistic regression analysis. A stepwise entry method was applied with forward selection and backward elimination to ensure duplication of findings. The accuracy of the model was verified with Hosmer-Lemeshow goodness-of-fit test. Survival analysis for each categorical variable was performed on MACE, all-cause mortality and first hospital admission separately using Kaplan-Meier survival curves and assessed visually and for statistical significance using the log-rank test. Multivariate Cox proportional hazards regression analyses were performed, for potential predictor variables, on their ability to predict MACE, with mortality and first hospital admission considered separately. A forward

selection and backward elimination method was applied using the Wald statistic with $p<0.15$. A p-value <0.05 was considered statistically significant.

5.3.7 Missing Data

Missing data were anticipated given the method of data collection. Sequential searches of multiple sources were performed by two investigators to minimise data loss. A missing data analysis was performed to determine patterns of 'missingness' for selected predictors **(Table 5.1).** Absent demographic and medical background variables were assessed to demonstrate a MCAR pattern and continuous potential predictor variables (LVEF and QRS duration) have a MAR pattern. Precise LVEF measurments were not always possible due to specific patient factors, the main example being patients having a large body habitus. QRS duration could not always be measured as an ECG and was not available for a small minority of patients, (due primarily to its unavailability with external centres referals). Multiple imputation was performed to compensate for the small amount of missing data. Logistic regression analysis and proportional hazards Cox regression analysis models utilised multiple imputation data sets. Complete case analysis was performed to confirm multiple imputation models. Absence of electrocardiographic data represented the lack of availability of the baseline trace for those particular patients in their records.

Table 5.1 Selected 'Pre-Implant' Predictors Missing Data.

	Missing (n,%)	Valid N	Complete Case Mean±SD
Age at Implant	0 (0.0%)	300	71.5±10.1
Gender	0 (0.0%)	300	
CRT Device	0 (0.0%)	300	
Upgrade Status	0 (0.0%)	300	
Aetiology	10 (3.3%)	290	
Diabetic Status	10 (3.3%)	290	
CKD	8 (2.7%)	292	
BBB Morphology	41 (13.7%)	259	
QRS duration (msec)	40 (13.3%)	260	157.3±25.3
LVEF (%)	71 (23.7%)	229	24.1±5.3

5.4. RESULTS

5.4.1 Patient Characteristics

During the period of study 376 implants were performed; 11 (2.9%) were excluded because biventricular pacing was not possible at the end of implant and 65 (17.8%) excluded due to lack of clinical response data at follow up, leaving 300 patients (**Figure 5.1**). The overall response rate was 52.7% (n=158). Baseline characteristics of the cohort and comparison between overall clinical responders vs non-responders is shown in **Table 5.2**.

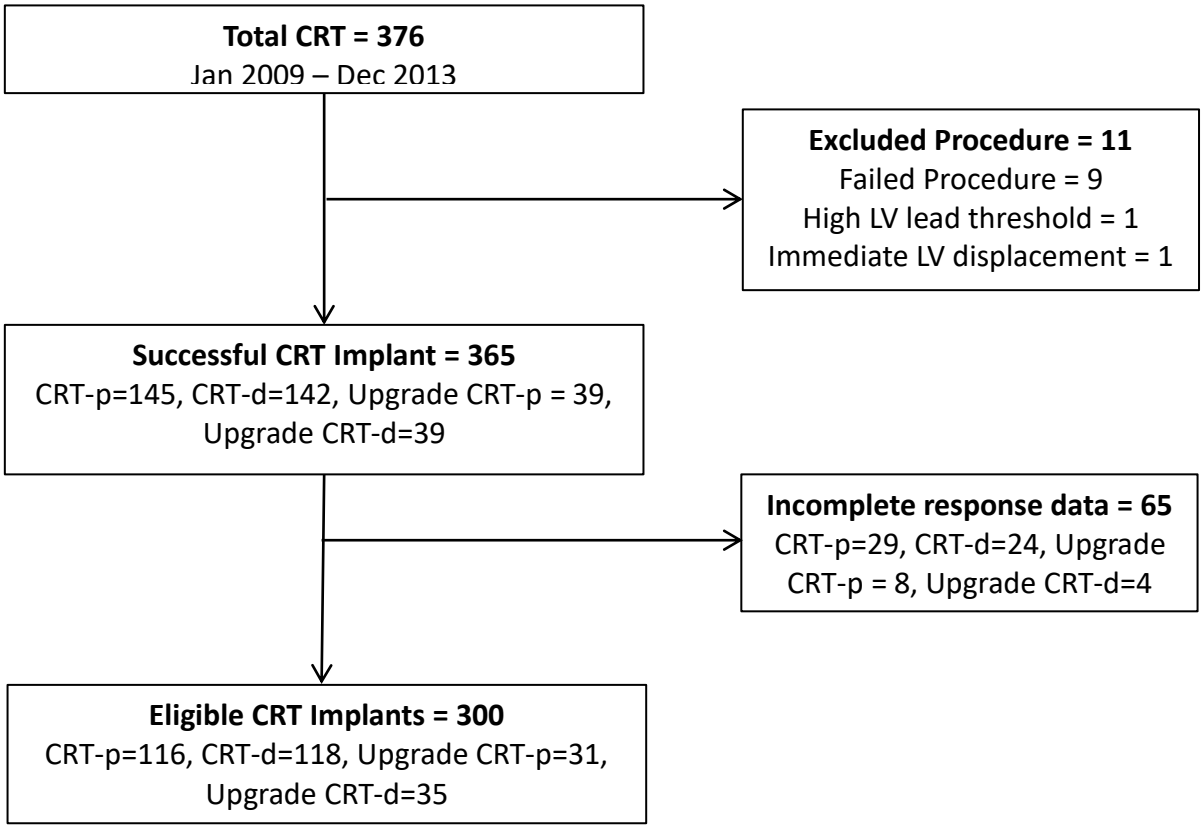


Figure 5.1 Screening and selection of all CRT implantations eligibile for study.

Table 5.2. Baseline characteristics of overall clinical responders vs non-responders.

	Total Cohort N=300	Responders N= 158	Non-Responders N= 142	P-value
Demographics				
Age (years, mean±SD)	71.5±10.1	69.9±10.7	73.3±9.1	<0.001
Male (n,%)	227 (75.7%)	119 (75.3%)	108 (76.1%)	0.98
Device				
CRT-D (n,%)	153 (51.0%)	87 (55.1%)	66 (46.5%)	0.17
Upgrade (n,%)	66 (22.0%)	27 (17.1%)	39 (27.5%)	0.04
Aetiology≠				
Ischaemic (n,%)	171 (59.0%)	93 (60.8%)	78 (56.9%)	0.58
Non-ischaemic (n,%)	119 (41.0%)	60 (39.2%)	59 (43.1%)	
Co-morbidities				
Prev-MI (n,%)≠	126 (45.0%)	71 (48.6%)	55 (41.0%)	0.24
CABG (n,%)≠	67 (24.1%)	38 (26.2%)	29 (21.8%)	0.47
Prev-PCI (n,%)≠	55 (20.5%)	28 (20.4%)	27 (20.6%)	1.00
Diabetes Mellitus (n,%)	75 (25.9%)	37 (24.2%)	38 (27.7%)	0.58
CKD (n,%)	103 (35.3%)	46 (29.7%)	57 (41.6%)	0.05
NYHA (n,%)				
I	4 (1.3%)	2 (1.3%)	2 (1.4%)	
II	24 (8.0%)	8 (5.1%)	16 (11.3%)	<0.01
III	246 (82.0%)	127 (80.4%)	119 (83.8%)	
IV	26 (8.7%)	21 (13.3%)	5 (3.5%)	
Electrocardiogram				
AF (n,%)	72 (28.0%)	33 (25.4%)	39 (30.7%)	0.42
QRS duration (msec), median (range))≠	154 (88-278)	157 (88-278)	154 (88-230)	0.77
LBBB (n,%)≠	186 (71.8%)	102 (75.0%)	84 (68.3%)	0.29
Echocardiogram				
LVEF (%, mean±SD)≠	24.10±8.30	23.70±8.60	24.97±8.01	0.15
Medications				
ACEi/ARB (n,%)	237 (82.0%)	126 (81.0%)	111 (82.9%)	0.79
Beta-blocker (n,%)	215 (73.9%)	108 (71.1%)	107 (77.0%)	0.31
MRA (n,%)	129 (51.4%)	66 (50.8%)	63 (52.1%)	0.94
LV Lead Position (n,%)				
Anteroseptal	1 (0.3%)	0 (0.0%)	1 (0.7%)	
Anterior	15 (5.0%)	5 (3.2%)	10 (7.0%)	
Anterolateral	32 (10.7%)	17 (10.8%)	15 (10.6%)	0.30
Lateral	194 (64.7%)	101 (63.9%)	93 (65.5%)	
Posterolateral	56 (18.7%)	33 (20.9%)	23 (16.2%)	
Posterior	2 (0.7%)	2 (1.3%)	0 (0.0%)	

≠ Based upon avaliable data

5.4.2 Implant details

Of the total cohort, 147 (49.0%) had CRTp and 153 (51.0%) CRTd implants; of the latter 103 (67.3%) had primary prevention and 50 (32.7%) secondary prevention indication for defibrillator implantation. There were 227 (75.7%) elective and 73 (24.3%) urgent CRT implants performed. Overall clinical response vs non-response in elective implants (52.9% vs 47.1%) and urgent implants (52.1% vs 47.9%) was not statistically different (*p*=1.0).Vascular access was cephalic vein with either one (n=140, 46.7%) or two (n=158, 52.6%) venous punctures. The RV lead was already in-situ in 46 (15.3%) cases. New RV leads were placed most commonly via the cephalic vein (n=177, 69.7%). There were 254 new RV leads placed, and they were deployed either at the RVA (n=239, 94.1%) or RV septum (n=13, 5.1%). Overall clinical response vs non-response was no different between different RV pacing sites (RVA: 55.6% vs 44.4% and RV septum: 69.2% vs 30.8%, *p*=0.16). A new RA lead was not placed if there was one already in-situ (n=34, 11.3%) or the patient was in permanent AF (n=52, 17.3%). RA leads were placed at the RAA (n=177, 82.7%) or RA free wall (n=36, 16.8%). The LV lead was most commonly placed via the axillary vein (n=184, 61.3%). The LV lead distal lead circumferential position is shown in **Table 5.2** with no statistical difference noted when evaluating overall clinical response. The LV lead axial position was basal (n=57, 19.0%) or mid-cavity (n=236, 78.7%) in the majority with only a few apical (n=7, 2.3%). There was no statistical difference demonstrated with overall clinical response (*p*=0.56).

There were 66 (22.0%) device upgrades to CRT within the cohort (**Figure 5.1**): 30 pacemakers upgraded to CRT-p (n=23 dual, n=7 single lead), 1 single lead ICD upgraded to CRT-p, 16 pacemakers upgraded to CRT-d (n=9 dual, n=7 single lead) and 19 ICDs (n=15 dual, n=4 single lead) upgraded to CRT-d. Upgrade status was significantly different between

overall clinical responders and non-responders (**Table 5.2**). The index cardiac device was compared with overall clinical response vs non-response with no significant difference found (*p*=0.082): single chamber pacemaker (14.8% vs 25.6%), dual chamber pacemaker (51.9% vs 46.2%), single lead ICD (33.3% vs 15.4%) and dual chamber ICD (0% vs 12.8%).

5.4.3 Clinical response

Overall there were 158 (52.7%) responders and 142 (47.3%) non-responders with a median (range) time to assessment of 12.0 (0.02-68.2) months. In the overall cohort older age, presence of device upgrade and CKD were significantly more common in the non-responders (**Table 5.2**).

Acute response was definable for 247 patients: 153 (61.9%) responders and 94 (38.1%) non-responders. **Table 5.3** provides the baseline characteristics of the cohort where an acute response was definable. The median (range) follow-up to acute response assessment was 1.4 (0.02-3.0) months. There were no significant differences in characteristics between acute responders and non-responders.

Long-term response was determined for 238 patients with 116 (48.7%) responders and 122 (51.3%) non-responders; the median (range) follow-up was 15.2 (3.1-68.2) months. **Table 5.4** demonstrates baseline characteristics of these patients. Long-term response cohort characteristics were similar to the overall cohort. The two variables that demonstrated statistical significance between clinical responders and non-responders were older age (68.9±10.68 vs 72.9±9.40, *p*<0.01) and presence of CKD (27.0% vs 41.5%, *p*=0.03). Presence

of device upgrade showed a significant difference for overall response between responders and non-responders, but did not reach statistical significance for long-term response.

5.4.4 Predictors of Clinical Response

Table 5.5 demonstrates increasing age was an independent predictor of overall clinical response. Age at implant was demonstrated to be the strongest predictor with an OR of 0.96 (p=0.002, CI 0.94-0.99), representing a 4% decreased chance per year of age of having a clinical response. Increasing time from implant to assessment was observed to be an important confounder for predicting non-response (OR 0.99, p=0.03, CI 0.99-1.00). Upgrading devices to CRT was also suggestive of predicting non-response, but did not reach statistical significance (OR 0.57, p=0.05, CI 0.32-1.01). Complete case analysis verified the findings for the imputed analysis. QRS duration as a predictor in the cohort was evaluated by performing quadratic and logarithmic transformation as it was a non-normally distributed variable. These analyses did not demonstrate that QRS duration had the ability to predict clinical response. The limited numbers in the cohort may explain the associations that are seen as chance observations and do not rule out roles as predictors.

Table 5.3 Baseline characteristics acute response (≤12 weeks) response vs non-responders

	Total Cohort N= 247	Responders N= 153	Non-Responders N= 94	P-value
Demographics				
Age (years, mean±SD)	71.4±10.2	70.7±10.8	72.4±9.2	0.22
Male (n,%)	186 (75.3%)	115 (75.2%)	71 (75.5%)	1.00
Device				
CRT-D (n,%)	128 (51.8%)	80 (52.3%)	48 (51.1%)	0.97
Upgrade (n,%)	53 (21.5%)	33 (21.6%)	20 (21.3%)	1.00
Aetiology≠				
Ischaemic (n,%)	139 (58.4%)	60 (41.1%)	39 (42.4%)	0.95
Non-ischaemic (n,%)	99 (41.6%)	86 (58.9%)	53 (57.6%)	
Co-morbidities				
Prev-MI (n,%)≠	96 (42.1%)	60 (43.2%)	36 (40.4%)	0.79
CABG (n,%)≠	52 (22.8%)	31 (22.1%)	21 (23.9%)	0.89
Prev-PCI (n,%)≠	43 (19.5%)	24 (18.0%)	19 (21.6%)	0.52
Diabetes Mellitus (n,%)	63 (26.4%)	36 (24.7%)	27 (29.0%)	0.55
CKD (n,%)	81 (33.8%)	31 (33.7%)	50 (33.8%)	1.00
NYHA (n,%) I	2 (0.8%)	0 (0.0%)	2 (2.1%)	0.01
II	20 (8.1%)	9 (5.9%)	11 (11.7%)	
III	205 (83.0%)	127 (83.0%)	78 (83.0%)	
IV	20 (8.1%)	17 (11.1%)	3 (3.2%)	
Electrocardiogram				
AF (n,%)	63 (29.7%)	32 (25.0%)	31 (36.9%)	0.09
QRS (msec, median (range))≠	152 (88-230)	154 (95-206)	150 (88-230)	0.26
BBB (n,%)≠	155 (72.1%)	98 (73.1%)	57 (70.4%)	0.78
Echocardiogram				
LVEF (%, mean±SD)≠	23.9±8.3	23.3±8.2	25.0±8.5	0.20
Medications				
ACEi/ARB (n,%)	199 (82.9%)	75 (81.5%)	124 (83.8%)	0.78
BB (n,%)	179 (74.3%)	107 (71.8%)	72 (78.3%)	0.34
MRA (n,%)	107 (51.2%)	69 (53.5%)	38 (47.5%)	0.48
LV Lead Position (n,%)				
Anteroseptal	1 (0.4%)	0 (0.0%)	1 (1.1%)	0.50
Anterior	13 (5.3%)	7 (4.6%)	6 (6.4%)	
Anterolateral	28 (11.3%)	16 (10.5%)	12 (12.8%)	
Lateral	163 (66.0%)	105 (68.6%)	58 (61.7%)	
Posterolateral	41 (16.6%)	25 (16.3%)	16 (17.0%)	
Posterior	1 (0.4%)	0 (0.0%)	1 (1.1%)	

≠ Based on available data

Table 5.4 Characteristics long-term (>12 weeks) response vs non-responders.

	Total Cohort N= 238	Responders N= 116	Non-Responders N= 122	P value
Demographics				
Age (years, mean±SD)	71.0±10.22	68.9±10.68	72.9±9.40	<0.01
Male (n,%)	179 (75.2%)	87 (75.0%)	122 (51.3%)	1.00
Device				
CRT-D (n,%)	119 (50.0%)	62 (53.4%)	57 (46.7%)	0.36
Upgrade (n,%)	50 (21.0%)	20 (17.2%)	30 (24.6%)	0.22
Aetiology≠				
Ischaemic (n,%)	137 (59.3%)	69 (60.5%)	68 (58.1%)	0.81
Non-ischaemic (n,%)	94 (40.7%)	45 (39.5%)	49 (41.9%)	
Co-morbidities				
Prev-MI (n,%)≠	105 (45.9%)	56 (49.6%)	49 (42.2%)	0.33
CABG (n,%)≠	51 (22.5%)	25 (22.3%)	26 (22.6%)	1.00
Prev-PCI (n,%)≠	50 (22.6%)	28 (25.9%)	22 (19.5%)	0.32
Diabetes Mellitus (n,%)	58 (25.2%)	27 (23.9%)	31 (26.5%)	0.80
CKD (n,%)	80 (34.3%)	31 (27.0%)	49 (41.5%)	0.03
NYHA (n,%) I	4 (1.7%)	2 (1.7%)	2 (1.6%)	<0.01
II	17 (7.1%)	4 (23.5%)	13 (10.7%)	
III	198 (83.2%)	95 (81.9%)	103 (84.4%)	
IV	19 (8.0%)	15 (12.9%)	4 (3.3%)	
Electrocardiogram				
AF (n,%)	59 (28.1%)	26 (26.0%)	33 (30.0%)	0.62
QRS (msec, median (range))≠	155 (88-278)	160 (88-278)	154 (88-230)	0.82
LBBB (n,%)≠	149 (71.6%)	102 (74.5%)	73 (68.9%)	0.45
Echocardiogram				
LVEF (%, mean±SD)≠	25.0±8.48	23.1±8.2	25.1±8.7	0.10
Medications				
ACEi/ARB (n,%)	199 (85.4%)	101 (88.6%)	98 (82.4%)	0.24
BB (n,%)	179 (76.2%)	85 (74.6%)	94 (77.7%)	0.68
MRA (n,%)	108 (51.9%)	52 (51.0%)	56 (52.8%)	0.90
LV Lead Position (n,%)				
Anteroseptal	1 (0.4%)	0 (0.0%)	1 (0.8%)	0.26
Anterior	13 (5.5%)	4 (3.4%)	9 (7.4%)	
Anterolateral	28 (11.8%)	14 (12.1%)	14 (11.5%)	
Lateral	153 (64.3%)	72 (62.1%)	81 (66.4%)	
Posterolateral	41 (17.2%)	24 (20.7%)	17 (13.9%)	
Posterior	2 (0.8%)	2 (1.7%)	0 (0.0%)	

≠ Based on available data

Table 5.5 Univariate and Multivariate Logistic Regression Analysis of Potential Predictors of Overall Clinical Response (Pooled Multiple Imputation Model).

	Univariate Regression			Multivariate Regression		
Clinical Variable	**Odds Ratio**	**P-Valve**	**Confidence Interval (95%)**	**Odds Ratio**	**P-Valve**	**Confidence Interval (95%)**
Age at implant	0.97	<0.01	0.94 – 0.99	0.96	<0.01	0.94 - 0.99
Gender	0.96	0.88	0.57-1.63			
Device	1.41	0.14	0.90 - 2.22			
Upgrade Status	0.54	0.03	0.31 - 0.95	0.57	0.05	0.32 - 1.01
QRS Duration	0.87	1.00	0.99 - 1.01			
LBBB	1.42	0.20	0.84 - 2.42			
LVEF	0.98	0.21	0.95 - 1.01			
Aetiology	1.18	0.50	0.74 - 1.87			
Diabetes Mellitus	0.84	0.53	0.50 - 1.43			
CKD	0.60	0.04	0.37 - 0.97			
Days from Implant to Response Assessment	1.00	0.13	0.99 - 1.00	0.99	0.03	0.99-1.0

8.4.5 Predictors of Cardiovascular Outcomes

During the observation period there were 72 (24.0%) deaths at a median of 19.0 months (0.0-56.5). The number of first HF hospital admissions was 40 (13.3%) at a median of 7.4 months (0.6-50.6). The overall MACE event rate was 85 (28.3%) at median of 16.4 months (IQR 0.0-56.5). Analysis of individual pre-selected categorical variables using Kaplan-Meier survival curves (**table 5.6**) demonstrated that CKD was associated with significantly worse time-to-event rate for all-cause MACE (*p*<0.001), all-cause mortality (p<0.001) and first HF admission (*p*=0.03) (**figure 5.2: Panel A, B and C**). The presence of a cardiac device at implant that was to be upgraded was associated with a significantly worse survival curve for all-cause mortality (*p*=0.03) (**figure 5.3**), but was not significant for any other cardiovascular outcome measure. however, this association was not present when a Cox regression model was used **(table 5.7).** No other variables were identified on survival analysis as being associated with cardiovascular outcomes (**table 5.7**).

Multivariate Cox regression was performed for all clinical outcome measures on complete cases and multiple imputation models. Comparing both models demonstrated similar findings and supported the use of the multiple imputation model. Presence of CKD was the only variable demonstrated to predict cardiovascular outcomes following CRT implantation. CKD at implant inferred increased risk of MACE (HR 2.10, *p*=0.001, 95% CI: 1.23-3.19), all-cause mortality (HR 2.06, *p*=0.001, 95% CI: 1.22-3.46), and first HF admission (HR 1.95, p =0.036, CI: 1.05-3.63) (**Table 5.7, 5.8 and 5.9**). There were no other variables that predicted any defined cardiovascular outcomes.

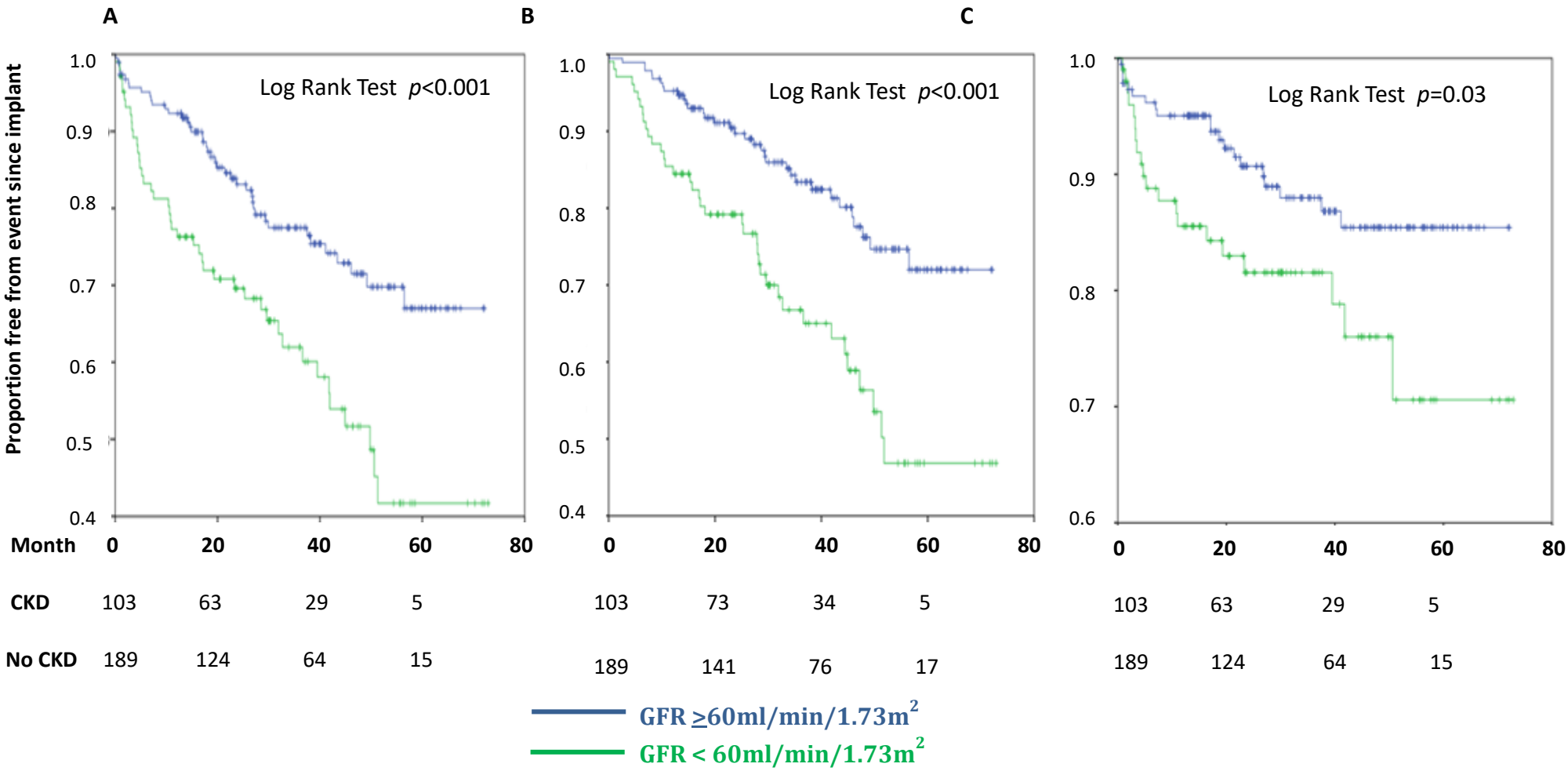


Figure 5.2CKD status at implant and time to MACE (A), all-cause mortality (B) and first HF hospitalisation (C)

Table 5.6 Log-rank (P-value) of Kaplan-Meier Curves

Clinical Variable	MACE	All-Cause Mortality	First HF Admission
Gender	0.61	0.21	0.98
Device	0.74	0.96	0.29
Upgrade Status	0.09	0.03	0.79
LBBB	0.86	0.86	0.73
DM	0.89	0.95	1.00
CKD	<0.001	<0.001	0.03
Ischaemic Aetiology	0.11	0.19	0.10

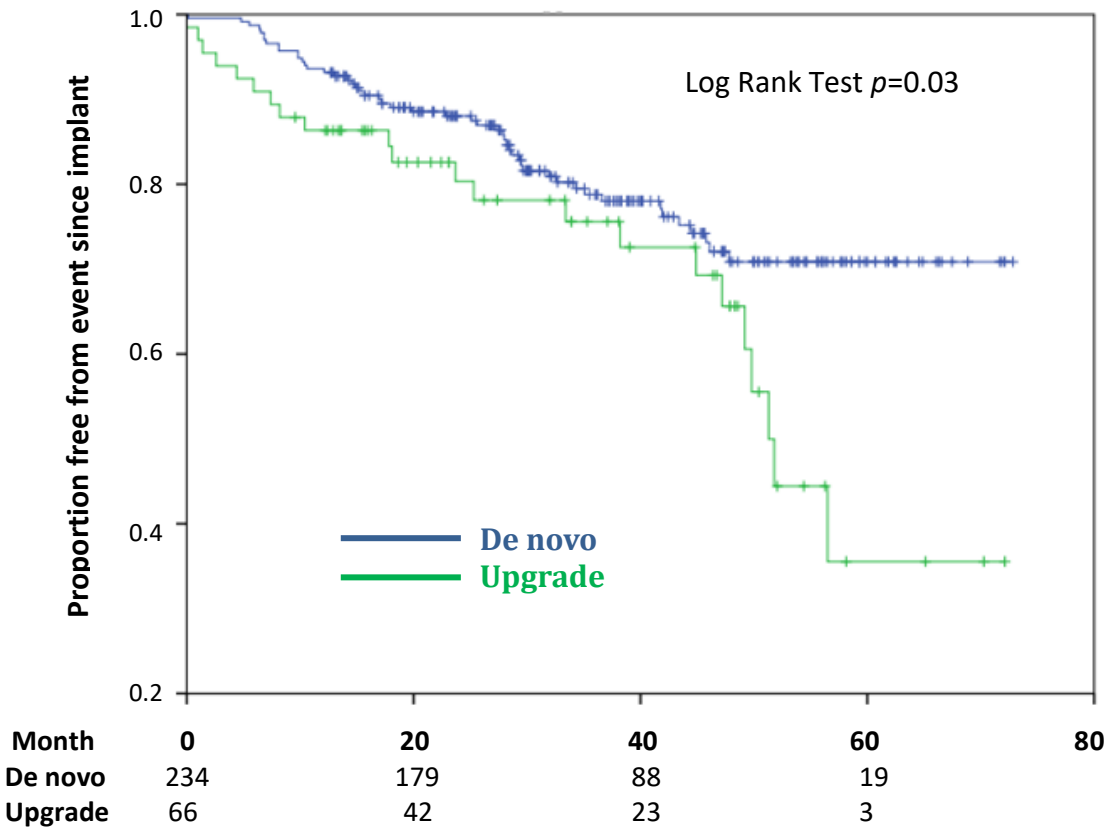


Figure 5.3 Upgrade device status at implant and time to all-cause mortality

Table 5.7 Univariate and Multivariate Proportional Hazards Cox Regression for MACE (Pooled imputation model)

	Univariate Regression			Multivariate Regression		
	Hazard Ratio	**P-Valve**	**Confidence Interval (95%)**	**Hazard Ratio**	**P-Valve**	**Confidence Interval (95%)**
Age at implant	1.01	0.31	0.99-1.04			
Gender	1.14	0.63	0.66-1.96			
Device	1.10	0.72	0.65-1.88			
Upgrade Status	0.83	0.50	0.49-1.41			
QRS Duration	1.00	0.49	0.99-1.01			
LBBB	1.02	0.93	0.59-1.77			
LVEF	0.99	0.58	0.96-1.02			
Ischaemic	0.69	0.21	0.39-1.23	0.73	0.19	0.46-1.17
Diabetes Mellitus	1.16	0.58	0.69-1.93			
CKD	1.92	0.001	1.22-3.00	2.10	0.001	1.23-3.19

Table 5.8 Univariate and Multivariate Proportional Hazards Cox Regression for all-cause mortality (Pooled imputation model)

	Univariate Regression			Multivariate Regression		
	Hazard Ratio	**P-Valve**	**Confidence Interval (95%)**	**Hazard Ratio**	**P-Valve**	**Confidence Interval (95%)**
Age at implant	1.02	0.10	0.99-1.05	1.03	0.06	1.00-1.06
Gender	1.51	0.19	0.82-2.80	1.58	0.14	0.87-2.79
Device	0.80	0.44	0.45-1.41			
Upgrade Status	1.30	0.36	0.74-2.27			
QRS Duration	1.00	0.50	0.99-1.01			
LBBB	1.10	0.77	0.59-2.03			
LVEF	0.98	0.24	0.95-1.01			
Ischaemic	1.28	0.45	0.68-2.40			
Diabetes Mellitus	0.84	0.53	0.48-1.47			
CKD	2.167	0.001	1.33-3.54	2.06	0.001	1.22-3.46

Table 5.9 Univariate and Multivariate Proportional Hazards Cox Regression for first HF Hospitalisation (Pooled imputation model)

	Univariate Regression			Multivariate Regression		
	Hazard Ratio	**P- Valve**	**Confidence Interval (95%)**	**Hazard Ratio**	**P-Valve**	**Confidence Interval (95%)**
Age at implant	1.01	0.65	0.97-1.04			
Gender	0.96	0.92	0.45-2.05			
Device	1.17	0.71	0.52-2.59			
Upgrade Status	1.02	0.97	0.46-2.27			
QRS Duration	1.00	0.69	0.98-1.01			
LBBB	1.21	0.66	0.53-2.74			
LVEF	1.01	0.78	0.96-1.05			
Ischaemic	1.54	0.34	0.64-3.72	1.72	0.13	0.86-3.45
Diabetes Mellitus	0.92	0.83	0.44-1.92			
CKD	1.92	0.05	1.00-3.71	1.95	0.04	1.05-3.63

5.4.6 Chronic Kidney Disease

CKD status was available in hospital coding data for 292 patients (97.3 %) at the time of CRT implantation. A recorded set of renal function blood tests were available at the time of implant for 194 patients (64.7%) of the cohort. These tests were performed at a mean ± SD of 5.7±9.4 weeks before CRT implantation. Blood results were available for 193 (66.1%) patients who had coding data available. Calculated GFR matched the coding categorisation in 74.6% of cases. There were 78 (86.7%) patients in CKD stage 3 (30-59 ml/min/1.73m^2)[239] with an estimated GFR 46.0±8.5 ml/min/1.73m^2. There were 11 (12.2%) patients in stage 4(15-29 ml/min/1.73m^2)[239] with a mean GFR of 25.4±3.6 ml/min/1.73m^2. Moreover there was only one patient in stage 5 (<15 ml/min/1.73m^2)[239] and they had a GFR of 14.7 ml/min/1.73m^2. The calculated GFR questioned the reliability of hospital coding data as a method for defining CKD status in a small number of cases. The difference between the biochemical and coding data may be accounted for by the patient's condition at the time of the blood sampling. An analysis was performed on the complete data set for patients with calculated GFR and it showed GFR did not show any statistical difference overall in clinical responders and non-responders. Those patients whom had CKD based upon calculated GFR had a significantly higher MACE rate; 36.7% vs 23.1% (p=0.001). These results support the prediction models utilising hospital coding data. Moreover a significant proportion of patients had missing blood test results, which may have weakened the analysis.

5.5 DISCUSSION

Non-response to CRT remains a significant problem for patients, healthcare providers and the wider society, despite implanted patients meeting international criteria.[234] Poor response has been linked to several factors; pre-implant, procedure and post implant. The

focus is now on patient selection prior to CRT implantation and the predictors that have shown some strength as predictors of response or cardiovascular outcome. There is often inconsistency reported in the literature regarding potential predictors (**Table 1.3**). A substantial contributor to the inconsistency in the literature is the lack of consensus on a definition of CRT response (**Table 1.4**).[1] Several studies utilise MACE definitions and others use specific clinical/echocardiographic combination definitions.[1] Importantly, definitions are often non-comparable producing different proportions of responders/non-responders when different definitions are applied to the same cohort.[1] Correlation is often poor, especially between clinical and echocardiographic definitions.[1] Ultimately, this makes comparison of data challenging, especially given varying study designs. Interpretation of our results has to be performed within this context. However, this small retrospective study does allow the best opportunity to examine variables that may be particularly important in our centres hetrogenous CHF population.

Increasing age at implant was the most significant predictor of overall (OR 0.96, p=0.002, CI(95%) 0.94-0.99)and long-term (OR 0.96, *p*=0.004, CI 0.93-0.99) clinical response in our study. Interestingly, when examining acute response alone, age at implant was not a statistically significant predictor. These observed patterns seem somewhat intuitive. However, no other cohort studies have demonstrated increasing age at implant predicts response or MACE. This study represents an older population, with most other studies having mean age in the low sixties.[38,65-67] Increasing age at implant within the study cohort predicted overall and long-term non-response.

Several patient parameters, including baseline ECG features, have been examined in multiple cohorts and post-hoc RCT analyses to determine their value in estimating risk stratification of patients undergoing CRT. QRS duration is considered one of the strongest predictors of response and forms a central part of implant criteria.[234] Cleland *et al*,[39] in the large meta-analysis previously described, demonstrated that only QRS duration added value in predicting cardiovascular outcomes. Improved cardiovascular outcomes in CRT patients increased in magnitude at QRS duration >132msec. The benefit reached a plateau beyond 180msec for composite outcome alone.[39] BBB morphology trended towards worse outcome for non-LBBB, but these were not statistically significant.[39] Interestingly Hsu *et al,*[38] in a post-hoc analysis of MADIT-CRT (trial not included in Cleland *et al*[39] meta-analysis), demonstrated QRS duration ≥150msec predicted an echocardiographic response (top quartile LVEF change at 12 months) based upon best subset regression analysis. Hsu *et al*,[38] demonstrated that only LBBB on ECG decreased the chances of a cardiovascular outcome occurring (all-cause mortality or non-fatal HF) within their best-subset. Lin *et al,*[66] in a single centre retrospective cohort study identified the only feature on baseline ECG that predicted echocardiographic response was LBBB morphology on multivariate regression analysis. Several single centre cohort studies, including this study, did not demonstrate any association between QRS duration or morphology and response or cardiovascular outcome on multivariate analysis.[65,67] The observation that QRS duration and/or BBB morphology in this cohort study did not predict response was unexpected. Quadratic and logarithmic transformation was attempted on QRS duration to see if data distribution was impacting the predicting ability of the variable. The ECG variable suffered from a degree of missing data and that was recorded in patient records tended to be non-specific. Repeat measurements were performed of available data, yet no association was observed. The missing data is the

likely reason why this variable was demonstrated to be a predictor of CRT response. Most importantly the study had very small numbers and is the likely reason why this association was not seen, even though it is well described in the literature.

Patient specific variables vary in their significance in predicting response to CRT in the literature. Cleland *et al*,[39] determined that no specific background clinical factor predicted cardiovascular outcome in those benefitting from CRT. Hsu *et al*,[38] determined that three background factors predicted echocardiographic response: female gender, body mass index <30kg/m^2 and no previous MI.[38] Importantly, these same factors did not predict cardiovascular outcomes within the same post-hoc analysis of MADIT-CRT. Gender was not shown to be a predictor of response within this study. Several other studies examined gender and did not demonstrate statistical significance for predicting response or outcome (**Table 1.3**).[67] Consistently amongst all cohort studies, females are under-represented including in this study. Body mass index was not examined within this study, due to absence of this specific baseline data in the clinical records. Several studies have commented on the importance of non-ischaemic aetiology (**Table 1.3**). Shanks *et al*,[65] demonstrated ischaemic aetiology increased the chance of non-response significantly.[65] This study did not demonstrate any significance of aetiology as a predictor of response or MACE, which is supported by several other studies.[39,66]

CKD status (eGFR <60ml/min/1.73m^2) present in a patient pre-CRT implant was shown in our study to infer a 2-fold increased risk of MACE (HR 2.10, p=0.001, CI(95%) 1.23-3.19) and all-cause mortality (HR 2.06, p=0.001, CI(95%) 1.23-3.46). These results replicate several

other studies, which show CKD status predicts worse cardiovascular outcomes for patients undergoing CRT. Hoke *et al*,[300] observed in a small prospective cohort study that patients with CKD stage 4 undergoing CRT had only a 30% echocardiographic response (≥15% reduction LVESV at 6 months), much lower than is traditionally reported. However, cardiovascular outcomes were reported to be better than patients with CKD stage 4 having an ICD implant alone. Hoke *et al*,[300] therefore demonstated an improvement in renal function in patients who had a CRT implanted with CKD stage 4.[300] Seperately Lin *et al,*[237] in a large retrospective cohort study demonstrated CKD status inferred an increased risk (OR 1.61, $p<0.01$, CI (95%) 1.2-2.3) of long-term cardiovascular events (all-cause mortality and heart transplantation). Furthermore, Bogdan *et al,*[238] undertook an observational cohort study (n=179) of all CRT implants at a single centre and identified CKD (GFR<60ml/min/1.73m^2) strongly predicted all-cause mortality (HR 2.03, CI (95%)1.14–3.61, p=0.01) but did not predict 1 year functional response (composite of change in NYHA, QoL and 6MWT) (HR: 0.74; CI(95%): 0.38–1.43; p=0.37). We also observed CKD status did not predict non-response in our multivariate model, although the univariate analysis suggested it did.

CHF patients with CKD are well known to have poor cardiovascular outcomes,[301] which is also reflected in higher event rates in patients with eGFR <60ml/min/1.73m^2 undergoing CRT.[237,238] The increased risk of poor cardiovascular outcomes following CRT implantation in patients with CKD stage 3 or above does not appear to be related to adverse cardiac remodelling. Lin *et al*[237] observed patients with and without CKD at implant had improvement in LVEF and LV end diastolic dimensions. This observation is somewhat

unexpected considering advancing CKD has previously been observed to be associated with maladaptive LV hypertrophy and increased myocardial fibrosis, causing diastolic dysfunction.[302] However, these particular observations were made on patients in end stage renal failure on dialysis[302] unlike Lin *et al.*[237] CRT causes reverse remodelling of the LV in patients with and without advancing CKD, however the poor cardiovascular outcomes rates remain unchanged. This suggests that poor cardiovascular outcomes in CHF patients undergoing CRT with advancing CKD is not completely related to the degree of adverse cardiac remodelling. Whether the mechanism for poor cardiovascular outcomes in these patients is related to the coronary artery disease risk burden or an alternative mechanism is not clear.

Interestingly, CKD status in several studies[238] including ours demonstrates a strong ability to predict poor cardiovascular outcomes, but not functional/symptomatic response. This is partly because functional response poorly correlates with reverse remodelling, but also suggests there is a lack of relationship between these two factors. An additional reason which needs to be considered is that GFR is calculated by more variables than just serum creatinine. The modification of diet in renal disease equation is used in our study accounts for multiple variables; age, gender, serum creatinine, ethinicity, serum urea, serum albumin and body surface area.[239] Therefore all these variables may be having an important influence on CKD stage/GFR as a predictor of cardiovascular outcomes. Age at implant has already been discussed a possible determinant of functional response and this may influence GFR as a variable. Body composition is also discussed in **chapter 10** as a potential important variable in CHF and following CRT.

Comparative analysis of lead positions within our study demonstrated a statistically significant difference between RV lead position in the overall clinical response. Proportionally more responders had the RV lead at the septum than RV apex; however, only a small proportion of patients had leads placed at the RV septum, but the statistical difference was an interesting observation. Lead position was not included in the logistic regression model as it was not a pre-defined variable. A small pilot study has suggested adjustment in RV lead position can enhance acute haemodynamic CRT response.[303] Right heart lead position does pose an interesting question in predicting response but requires further study.

5.5.1 Study Limitations

Our study was retrospective and performed in a single-centre, which reflected a relatively small number of patients across a five year period. This study was not powered for the study outcomes, which importantly means absolute conclusions, cannot be drawn from the results of this study. There was a degree of missing data, which may have influenced the associations that were reported. The echocardiographic data had a significant proportion of missing data which was due to the lack of availability of external centres scans. Specific measurements were not performed on the baseline scan due primarily to scan quality, which can be influenced by body habitus. ECG reporting was not always correct and not all baseline traces were available. The availability of an ECG was generally related to where a patient was referred from. Both these factors represent selection bias for these particular variables. Repeat measurements of all echocardiograms and ECGs were performed independent of the original reports, this minimised reporting bias, but could be only

performed if the information was available. The response criteria utilised was based upon NYHA classification during the latest review; this is one of the most consistently used clinical response measures,[304] however poorly correlates with other established definitions.[1] Assessment on NYHA class was based upon recorded findings and consensus of three clinical reviewers, though blinded to each others assignments, a reporting bias cannot be completely removed**.**

5.5.2 Informing Prospective Cohort Study

The first stage of this research thesis was to understand the HF population dynamics that is served by our implantation centre. The Arden Cardiac Network (**figure 5.1**) region represents a diverse population in both ethnicity and socio-economic status. Our retrospective study was a complete analysis of all implants performed since th service began. Firstly our study demonstrated the heterogeneity of our local HF population having CRT implantations. Secondly our study demonstrates the challenge of using only change in NYHA class as a definition and on reflection a more robust measure of functional response was required. Thirdly, this study has partially informed the selection of pre-implant variables for the planned prediction model for the phase II proof of concept study. Age at implant and CKD status have proven to be the most useful variables in this study for predicting clinical response and cardiovascular outcomes respectively. However, other variables have proven more robust at predicting response and cardiovascular response in the literature. The prospective study prediction models will account for these variables, in particular resting ECG QRS duration and BBB morphology.[38,39]

5.6 CONCLUSION

In this real world heterogeneous cohort retrospective observation study increasing age at implant was associated with poor overall clinical response. It was also observed CKD status predicted long-term non-response and strongly predicted future MACE and all-cause mortality. These variables will be used to inform the planned prediction model for the phase II proof of concept study. Important variables including QRS duration and BBB morphology were not observed in this cohort to predict clinical response and this is likely due to the degree of missing data. This study also highlights the challenge of using a single metric as the definition of response. These factors have informed the planning and performance of the phase II study

5.7 PUBLICATIONS

*The work in this chapter has been produced into two publications and several conference abstracts. Data from this chapter has been presented at several national and international conferences including the American Cardiology Congress, San Diego March 2015 and British Cardiovascular Society Conference June 2016 (**Appendix R**). This cohort also formed part of a study examining same-day complex cardiac device implantation[224], which was submitted and published in The American Journal of Cardiology on the May 2016 (**Appendix S**). Furthermore a smaller study was conducted on the long-term follow-up of the epicardial lead placements,[305] which formed a small part of this cohort. This article was published in the PACE journal August 2016 (**Appendix S**).*

Chapter Six

THE CHARACTERISATION OF CIRCULATING BIOMARKERS BEFORE AND AFTER CRT IN PATIENTS WITH CHF AND THEIR ROLE IN PREDICTING RESPONSE:

THE COVERT-HF STUDY

6.1 INTRODUCTION

HFrEF is characterised by adverse cardiac remodelling resulting in a progressive reduction in LVEF and poor cardiovascular outcomes.[22,23,31] CRT has revolutionised the treatment of HFrEF patients with dyssynchrony refractory to optimal medical therapy by demonstrating a consistent reduction in mortality and morbidity.[22,23,31] Reverse cardiac remodelling has also been shown to be induced by CRT.[31,306] Unfortunately, no functional improvement is observed in 20-40% of HFrEF patients following CRT implantation.[22,23,26,27,60]

Maladaptive processes of neurohumoral activation, cardiac ECM remodelling, pro-inflammatory changes and myocardial wall stress are central mechanistically to the development and progression of HFrEF.[94] Altered ECM turnover in HFrEF is directly associated with adverse cardiac remodelling[113] and biomarkers of collagen synthesis (PINP,PICP and PIIINP)[125-127] and degradation (ICTP or CITP)[119,126] have been shown to be associated with poor HF outcomes. As key regulators of ECM turnover, matrix metalloproteinases (MMP-2 and -9)[135,136] have also been implicated as biomarkers of HF diagnosis and prognosis. GDF-15 is a member of the transforming growth factor-β cytokine superfamily involved in the regulation of cell survival, proliferation and differentiation that is associated with poor HF outcomes[110] and has been suggested as a potential predictor of CRT response.[112]

MiRNAs are short (20-22 nucleotides) endogenous non-coding ribonucleic acids that have been attracting much interest as key regulators of gene expression, including the cardiovascular system.[139,140] MiRNAs are readily measured in blood plasma and serum, and several circulating miRNAs have been shown to be dysregulated in cardiovascular disease,

suggesting potential use as biomarkers. [139,160,197] Specifically, miR-21, -30d[199], -122[173,180], -133a[181,183], and -210[149] have been demonstrated to be dysregulated in adverse cardiac remodelling and HFrEF.[139]

Markers of collagen turnover have previously been studied to establish their value as predictors of CRT response, but outcomes have thus far been inconsistent;[119,125,126,244,294] miRNAs are implicated as a novel set of biomarkers with variation reported in HFrEF,[139,188] but limited information is available on their ability to predict CRT response.[197,199] MiR-30d in particular has been proposed to be mechanistically related to LV wall stress and to predict cardiac remodelling following CRT implantation.[199]

6.2 AIM AND OBJECTIVES (Chapter 2)

This proof-of-concept prospective observational study principle aim was to evaluate the ability of a panel of potential predictor variables including novel ECM and miRNA biomarkers to predict functional response of HFrEF patients who meet CRT implantation criteria to respond long-term.

Secondary aims and objectives are to examine and characterise specific ECM and miRNA behaviour in HFrEF patients undergoing and following CRT implantation. Furthermore to examined the differences between systematic and CS expression in specifically defined ECM and miRNA biomarkers at the time of CRT implantation. Moreover to correlate biomarker expression overtime with functional and echocardiographic variables.

6.3 METHODOLOGY (Chapter 3.5)

The COVERT-HF study is a proof-of-concept prospective observational study of unselected HFrEF patients undergoing CRT implantation at a single tertiary centre between November 2013 and June 2015. The trial was registered at clinicaltrials.gov under reference NCT02541773 (**Appendix F**). Participants were eligible if they met the NICE[17,51] criteria for resynchronisation therapy in HF patients (LVEF≤35% on OMT≥3/12; NYHA I with QRS ≥150msec or NYHA II-IV with QRS ≥150msec or QRS 120-149msec and LBBB). Patients in AF or requiring a cardiac device upgrade (LVEF<35% and ventricular pacing >40%) were also included in the study to reflect clinical practice.[17,56] The NICE CRT guidelines were updated (TA120) in 2014 and the inclusion criteria were updated at this time accordingly. Patients were excluded if there was a recent acute coronary syndrome or acute heart failure decompensation event (<6 weeks), end-stage renal disease (on renal replacement therapy), significant cognitive impairment or a terminal illness unlikely to have a survival greater than a year. Post-implantation exclusions were applied in case of procedure failure or complications resulting in unsuccessful biventricular pacing (e.g. lead displacement/ non-avoidable phrenic nerve pacing).

Each participant underwent a pre-implantation visit and two follow-up visits at 6 weeks and 6 months post-implantation. Participants underwent clinical assessment (NYHA class), MLHFQ, 6MWT, transthoracic echocardiography, resting 12-lead ECG and blood sampling at all research attendances. All follow-up study visits coincided with routine CRT device checks. All participants provided written informed consent. The study was conducted in accordance with the 1975 Declaration of Helsinki and was approved by the South Birmingham Regional Ethics committee (13/WM/0355).

6.3.1 Study Outcomes (Chapter 3.5)

The primary outcome measure for the study was the patients' functional response status. Functional responders were defined as those whom survived, did not undergo heart transplantation and achieved two out of three response criteria (↓≥1 NYHA, ↑≥10% 6MWT distance, ↓MLHFQ score>5) at 6 months follow-up. Secondary outcomes were categorised as echocardiographic response, defined as a ≥15% reduction in LV end-systolic volume at 6 months, and major adverse cardiovascular events (MACE), defined as a composite of all-cause mortality and first HF hospital admission.

6.3.2 Device Implantation (Chapter 3.2)

CRT devices were implanted at UHCW according to local standard operating procedures.

6.3.3 Transthoracic Echocardiography (Chapter 3.5)

All participants underwent transthoracic echocardiography (Vivid 7, GE Healthcare, Horten, Norway) examination for LV volumetric assessment. Each echocardiogram was performed on the same machine by the same nationally accredited operator for each study visit. Grey-scale two dimensional images were obtained in the standard parasternal (long and short axis) and apical (2, 3, 4 chamber) view to allow LV volumetric assessment according to published guidelines.[201] All measurements were analysed offline (EchoPac, GE Healthcare, Horten, Norway). LV ejection fraction was estimated using the biplane method of discs (modified Simpsons method).[201] A blinded inter-rater study was conducted on all scans of a randomly selected 20% of the study participants. The inter-rater study for available comparative measures demonstrated a strong correlation between rater measurements (r^2=0.90, p<0.01). Paired measurements were not significantly different between both raters

when compared with a paired T-test (*p*=0.90). Bland-Altman limits of agreement were calculated to be 20.7%.

6.3.4 Blood Sampling and Laboratory Analysis (Chapter 5.5 and Chapter 6)

Peripheral venous sampling of blood was performed following two hours of fasting and one hour of rest, using EDTA as anticoagulant. Serum and plasma was prepared by single centrifugation at 3500 RPM for 10 minutes, followed by storage at -80 ^{o}C until analysis. Peripheral samples at implantation were taken the morning of the implant. CS sampling was performed in half the cohort (n=26) during CRT implantation upon cannulation of the CS. Clinical laboratory measurements were performed according to standard hospital procedure. ECLIA analysis for NT-pro-BNP and hs-TnT were performed in the local laboratory.

Plasma levels of PINP and CTx were determined using an ECLIA Cobas ®8000 modular analyser (Roche Diagnostics, Basel, Switzerland). The immunoassays inter and intra assay of precision for P1NP is <3.0%, and for CTx was <2.5%. The assays manufacturer calculated measures and were locally validated according to the validated international Clinical and Laboratory Standards Institute EP05-A3 protocol.[271]

Sandwich ELISA were used to determine plasma levels of GDF-15, MMP-2 and -9 (R&D Systems Inc, Minneapolis, Minnesota, USA) and PIIINP (Cusabio, Wuhan, Hubei, China) according to the manufacturers' protocol. The inter-assay and intra-assay coefficient of

variability was calculated for each assay: GDF-15 (7.7% and ≤4.8%); MMP-2 (4.5% and ≤5.6%); MMP-9 (10.3% and <12.9%); PIIINP (5.1% and ≤7.2%).

MiRNA profiling was undertaken for MiR-21, -30d, -122, -133a, -210 and -486 using previously described methods.[277] MiR-486, being highly enriched in erythrocytes[307], was used as a marker for sample quality with regards to hemolysis.[308] In brief, total RNA including small RNA was extracted using the miRNeasy Mini kit (Qiagen, Hilden, Germany). An exogenous miRNA (cel-miR-39-3p) was added to the plasma prior to the extraction procedure to serve as a spike-in normalization control. MiRNA's were reverse-transcribed using the TaqMan® MicroRNA Reverse Transcription Kit and RT Megaplex™ Primer pools (Human Pools A v2.1 and B v2.0, Applied Biosystems®, Darmstadt, Germany) and further amplified using TaqMan PreAmp Master Mix and Megaplex™ PreAmp Primers (Primers A v2.1 and B v2.0, Life Technologies, Massachusetts, USA). TaqMan® miRNA assays (Applied Biosystems) and TaqMan® Universal PCR Master Mix, no AmpErase® UNG were used for quantitative real-time polymerase chain reaction (qPCR) of specified miRNAs on a ViiA7 Real-Time PCR System (Life Technologies). Relative quantification was performed using the 2-ΔΔCt method.[309] Hemolytic samples were excluded from the analysis.

6.3.5 Statistical Analysis (Chapter 3.8)

Statistical analysis was performed using SPSS, version 22.0 (IBM, Chicago, Illinois). Categorical variables were reported as frequency and percentages. Comparison analyses for categorical data were performed using the Chi-Squared or Fisher's Exact tests, dependent on appropriateness. Continuous data and the residuals save from the appropriate analyses underwent histogram plots for assessment of normality. Normally distributed data were

reported as mean ±SD and comparative analysis was performed using independent t-tests. Non-normally distributed data were reported as median (full range) and were compared using a Mann-Whitney U test. Paired continuous data were analysed with a paired t-test or Wilcoxon Signal Rank test, as appropriate. Non-normally distributed ECM biomarkers, NT-pro-BNP, GDF-15 and hs-TnT circulating biomarker data was transformed logarithmically. Fold change was calculated using the mean (responder/non-responders) cohort value when comparing two datasets. Variation in continuous variables over three time periods was analysed using either one-way analysis of variance (ANOVA) or Friedman test, respectively. Mixed between-within subjects analysis of variance was used to compare variation in continuous data in functional responders and non-responders over 6 months of observations. Bivariate correlation analysis either Pearson (parametric) or Spearman rank (non-parametric) estimators was performed between two continuous variables to explore relationships. Univariate logistic regression analysis was performed for functional response for pre-defined circulating biomarkers and established clinical variables. Those variables that achieved a p-value <0.20 were pooled as co-variants for multiple logistic regression. A high alpha was set on the basis of the clinical response definition. A stepwise entry method was applied with forward selection and backward elimination to ensure duplication of findings. The accuracy of the model was verified with a Hosmer-Lemeshow goodness-of-fit test. A p-value <0.05 was considered statistically significant. Detailed discussion on the statistical methods employed in the prospective study was provided in **Chapter 3.8**.

6.4 RESULTS

A total of 58 patients consented to participation in the study, of which 52 participants were included in the study. **Figure 6.1** provides an overview of participant selection and

functional response categorisation. Follow-up research visits occurred at 1.7±0.3 and 5.7±0.7 months respectively. There were 27 (59.1%) responders and 22 (44.9%) non-responders after 6 months follow-up.

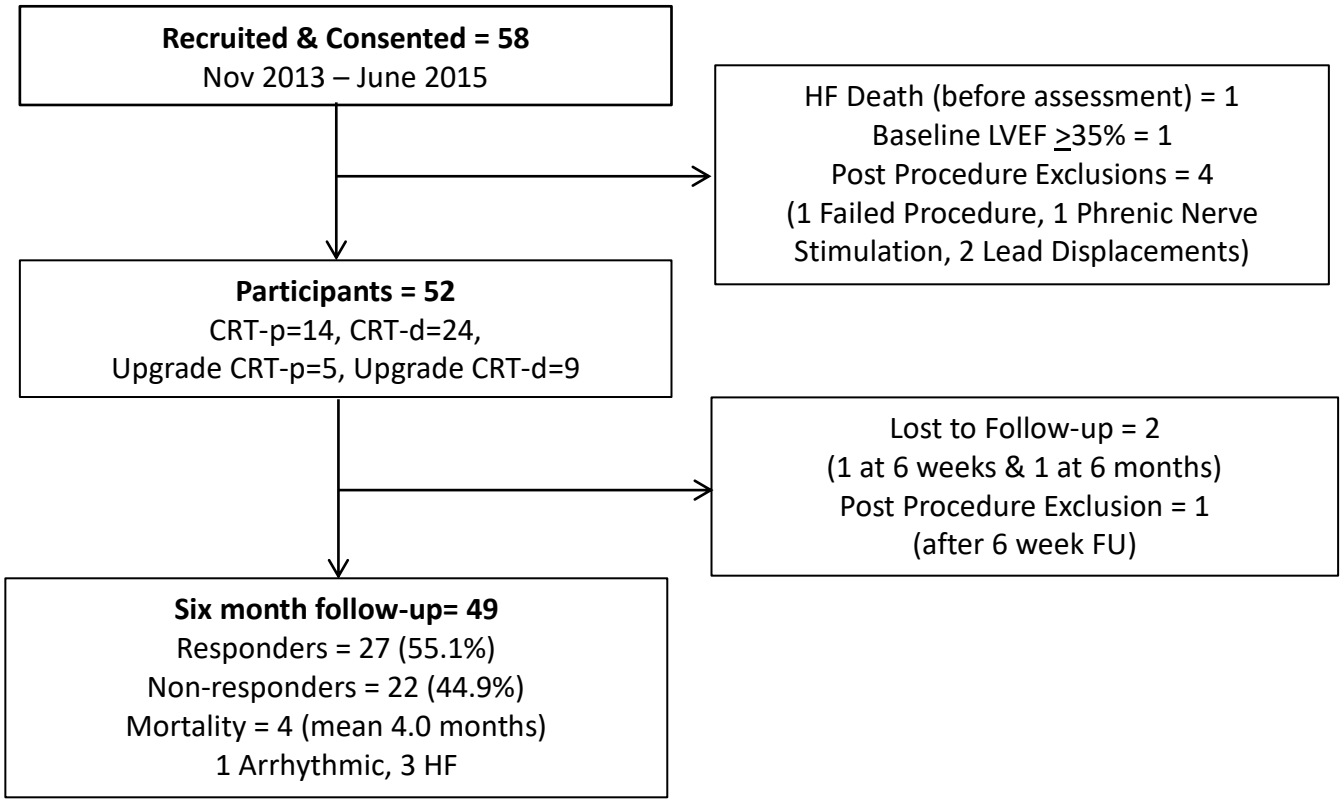


Figure 6.1 Patient recruitment, flow and outcomes.

6.4.1 Baseline Clinical Characteristics

Baseline characteristics of the study population and functional response rate are provided in **Table 6.1.** There were 43 (82.7%) males in the cohort with an average age of 72.4±9.4. with no significant difference between responders and non-responders. In the cohort, 30 (57.7%) had an ishaemic aetiology and 22 (42.3%) had a non-ischaemic cause, which did not significantly vary by response status. The median (range) QRS duration was 164.0 msec

(120.0-240.0) and 39 (75.0%) had Left Bundle Branch Block on the resting ECG at baseline. The QRS duration (168.0 msec vs 159.0 msec, *p*=0.11) and LBBB morphology (85.2% vs 63.6%), *p*=0.16) did not vary significantly between responders and non-responders respectively. However a trend for a wider QRS and presence of left bundle branch block morphology was observed more frequently in responders. The three patients lost in follow-up did not change the cohort characteristics.

Table 6.1 Baseline Characteristics

	Total Cohort=52	Responders=27	Non-Responders=22	p-value
Demographics				
Age (years, mean±SD)	72.4±9.4	72.0±10.4	73.0±8.5	0.74
Male (n,%)	43 (82.7%)	23 (85.2%)	17 (77.3%)	0.74
Device				
ICD: Primary Prevention (n,%)	26 (78.8%)	16 (88.9%)	9 (64.3%)	0.22
Upgrade (n,%)	14 (26.9%)	5 (18.5%)	9 (40.9%)	0.16
Aetiology				
Ischaemic (n,%)	30 (57.7%)	15 (55.6%)	13 (59.1%)	1.00
Non-ischaemic (n,%)	22 (42.3%)	12 (44.4%)	9 (40.9%)	
Co-morbidities				
History of Atrial Fibrillation (n,%)	28 (53.8%)	14 (51.9%)	12 (54.5%)	1.00
Diabetes Mellitus (n,%)	15 (28.8%)	5 (18.5%)	9 (40.9%)	0.16
COPD (n,%)	10 (19.2%)	3 (11.1%)	6 (27.3%)	0.28
CKD (n,%)	23 (44.2%)	11 (40.7%)	12 (54.5%)	0.5
NYHA (n,%)				
II	20 (38.5%)	11 (40.7%)	8 (36.4%)	
III	27 (51.9%)	15 (55.6%)	10 (45.5%)	0.25
IV	5 (9.6%)	1 (3.7%)	4 (18.2%)	
Routine Blood Markers				
eGFR (mL/min/1.73 m^2, median, range)	61.5 (25.0-130.0)	59 (25.0-130.0)	58.0 (26.0-99.0)	0.36
Haemglobin (g/dl, mean±SD)	134.4±13.5	136.6±14.1	132.5±12.5	0.28
NT-*pro*-BNP (pmol/L, median, range)	248.7 (53.0-4138.0)	207.0 (53.0-4138.0)	255.5 (67.0-547.0)	0.37
hs-TnT (ng/L, median, range)	26.5 (6.5-233.0)	26.4 (8.5-233.0)	27.6 (6.5-61.8)	0.78
Medications				
ACEi/ARB (n,%)	50 (96.2%)	26 (96.3%)	21 (95.5%)	1.00
BB (n,%)	44 (84.6%)	22 (81.5%)	21 (95.5%)	0.24
MRA (n,%)	34 (65.4%)	17 (63.0%)	14 (63.6%)	0.96
Aspirin (n,%)	24 (46.2%)	13 (48.1%)	9 (40.9%)	0.83
Clopidogrel (n.%)	6 (11.5%)	3 (11.1%)	3 (13.6%)	1.00
Prasugrel (n,%)	1 (1.9%)	1 (3.7%)	0 (0.0%)	1.00
Electrocardiogram				
Atrial Fibrillation (n,%)	19 (36.5%)	9 (33.3%)	8 (36.4%)	0.5
QRS duration (msec, median, range)	164.0 (120.0-240.0)	168.0 (146.0-240.0)	159.0 (120.0-210.0)	0.11
LBBB (n,%)	39 (75.0%)	23 (85.2%)	14 (63.6%)	0.16
QoL Score (mean±SD)	48.5 (8-101)	50 (9-86)	48.5(8-101)	0.77
6MWT (M,mean±SD)	238.8±130.6	237.6±130.5	239.4±127.4	0.95

Echocardiogram				
LVIDD (mm, mean±SD)≠	61.7±10.1	62.9±11.2	60.2±9.0	0.4
LVESV (ml,median,range)≠	111.5 (49.4-219.3)	119.3 (49.4-268.7)	110.2 (56.0-169.4)	0.41
LVESV_BSA (ml,mean±SD)≠	61.3±21.5	65.7±25.8	56.6±16.3	0.26
LVEF (%, mean±SD)≠	24.3±8.0	24.1±7.9	24.4±8.7	0.91
LV Lead Circumferential Position (n,%)				
Anterior	5 (9.6%)	2 (7.4%)	3 (13.6%)	0.64
Anterolateral	3 (5.8%)	2 (7.4%)	1 (4.5%)	
Lateral	28 (53.8%)	13 (37.0%)	13 (59.1%)	
Posterolateral	16 (30.8%)	10 (37.0%)	5 (22.7%)	
LV Axial Lead Position (n,%)				
Basal (n,%)	29 (55.8%)	15 (55.6%)	11 (50.0%)	0.7
Mid-Cavity (n,%)	23 (44.2%)	12 (44.4%)	11 (50.0%)	

6.4.2 Baseline Biomarker Levels

Table 6.2 shows baseline levels of the ECM biomarkers and miRNA panel for the total cohort and by functional response status. There was no significant difference in expression of ECM biomarkers of collagen synthesis or degradation between functional responders and non-responders at baseline. CTx were observed to be more highly expressed in responders than non-responders, but did not reach statistical significance (0.48 ug/L (0.14-1.14) vs 0.31 ug/L (0.16-0.73), *p*=0.07)). The biomarker of myocardial stress GDF-15 was not demonstrated to have significant variation in expression between responders and non-responders (1.12 ug/L (1.12-10.29) vs 1.20 ug/L (2.75-5.95), *p*=0.42). MiRNA biomarker expression profiles were not observed to vary significantly between functional responders and non-responders, although a trend for changes in miR-133a was observed (fold change 0.65, *p*=0.08). MiR-486 was found to have no significant difference between responder and non-responders at baseline (fold change 1.16, *p*=0.76).

Table 6.2 Baseline Biomarker Levels for Functional Responders and Non-Responders

	Total Cohort=52	Responders=27	Non-Responders=22	p-value
PINP (ug/L, median, range)	40.0 (15.0-141.0)	43.0 (22.0-141.0)	38.0 (15.0-113.0)	0.53
CTx (ug/L, median, range)	0.40 (0.14-1.14)	0.48 (0.14-1.14)	0.31 (0.16-0.73)	0.07
PIIINP (ug/L,mean±SD)	1.02±0.39	1.09±0.35	0.94±0.43	0.11
MMP-2(ug/L, median, range)	277.3 (155.3-789.5)	258.8 (157.0-789.5)	323.5 (155.3-543.4)	0.13
MMP-9 (ug/L, median, range)	73.5 (13.6-254.1)	71.8 (13.6-254.1)	80.8 (13.6-254.1	0.47
GDF-15 (ug/L, median, range)	2.66 (1.12-10.29)	2.66 (1.12-10.29)	2.75 (1.20-5.95)	0.42
miR-21 (RQ, median, range)	0.8 (0.3-2.4)	0.77 (0.3-1.9)	0.82 (0.5-2.4)	0.72
miR-30d (RQ, median, range)	0.7 (0.2-2.55)	0.81 (0.2-2.55)	0.68 (0.29-2.27)	0.35
miR-122 (RQ, median, range)	0.50 (0.06-3.60)	0.45 (0.08-3.60)	0.69 (0.06-2.48)	0.27
miR-133a (RQ, median, range)	0.78 (0.01-4.52)	0.34 (0.01-4.52)	1.28 (0.01-3.79)	0.08
miR-210 (RQ, median, range)	0.76 (0.03-5.12)	0.57 (0.17-5.12)	0.79 (0.03-3.61)	0.36
miR-486 (RQ, median, range)	0.76 (0.17-3.14)	0.87 (0.17-3.14)	0.73 (0.21-2.48)	0.76

6.4.3 Effects of CRT on Cardiac Function and Biomarker Expression

The impact of implanting a CRT on functional variables, LV geometry and biomarker expression over 6 months was examined. Trends between functional responders and non-responders were compared. **Table 6.3** summarises all the changes during follow-up. **Figure 6.2** demonstrates the most significant changes (6MWT, LVEF, PINP and MMP-2) over the period or follow-up for changes over time and between groups. Quality of life scores and LVESV both improved following CRT implantation for responders and non-responders ($p<0.01$). MMP-9 was observed to have decreased expression in both groups over the follow-up period ($p=0.01$). MiR-122, a liver-specific miRNA' was the only miRNA to demonstrate significantly higher initial expression in non-responders compared to responders ($p=0.03$). MiR-486 expression trended towards a significant decrease in expression in both responders and non-responders ($p=0.05$).

Table 6.3 Behaviour of Functional LV Geometry and Circulating biomarkers Following CRT Implantation. Interaction between responders status and over time analysed.

	Responders			Non-Responders			Response P-value	Time P-value	Interaction P-Value
Parameter	**Baseline**	**6 weeks**	**6 months**	**Baseline**	**6 weeks**	**6 months**			
6MWT (M,mean±SD)	237.6±130.5	304.8±129.0	325.1±147.5	239.4±127.4	265.5±127.7	194.9±159.1	0.16	**<0.01**	**<0.01**
QoL Score (median, range)	50 (9-86)	19 (0-64)	21 (0-72)	48.5 (8-101)	28.5(0-73)	32 (0-83)	0.3	**<0.01**	**0.01**
LVEF (%, mean±SD)	24.1±7.8	29.5±8.9	33.4±10.8	24.5±8.7	30.4±9.6	33.1±7.1	0.66	**<0.01**	0.93
NT-*pro*-BNP (pmol/L, median, range)	207.0 (53.0-4138.0)	173.0 (27.0-3848.0)	116.0 (15.0-1690.0)	255.5 (67.0-547.0)	265.5 (49.9-896.0)	184.0 (30.9-1437.0)	0.59	0.96	0.27
Hs-TnT (ng/L, median, range)	26.4 (8.5-233.0)	24.0 (6.7-61.9)	23.1 (6.5-63.2)	27.6 (6.5-61.8)	29.2 (8.4-77.3)	31.2 (14.5-83.1)	0.84	0.82	0.35
PINP (ug/L, median, range)	43.0 (22.0-141.0)	48.0 (29.0-136.0)	53.0 (13.0-107.0)	38.0 (15.0-113.0)	39.0 (16.0-69.0)	41.0 (14.0-94.0)	**0.04**	**0.02**	0.41
CTx (ug/L,, median, range)	0.48 (0.14-1.14)	0.43 (0.08-1.06)	0.38 (0.07-1.30)	0.31 (0.16-0.73)	0.27 (0.16-0.59)	0.30 (0.10-0.79)	0.05	0.33	0.36
PIIINP (ug/L,mean±SD)	1.09±0.35	1.10±0.32	1.01±0.36	0.94±0.43	0.65±0.50	0.87±0.47	0.13	0.10	0.44
MMP-2(ug/L, median, range)	258.8 (157.0-789.5)	241.3 (163.9-695.2)	243.2 (159.8 (159.8-625.4)	323.5 (155.3-543.4)	295.1 (162.0(512.3)	299.8 (162.4-515.9)	0.51	**<0.01**	0.19
MMP-9 (ug/L, median, range)	71.8 (13.6-254.1)	52.3 (5.6-192.9)	61.3 (8.8-126.1)	80.8 (13.6-254.1	65.2 (29.5-129.6)	58.3 (16.9-143.3)	0.77	**0.01**	0.66
GDF-15 (ug/L, median, range)	2.66 (1.12-10.29)	2.69 (0.97-6.27)	2.34 (0.99-5.68)	2.75 (1.20-5.95)	3.06 (1.09-5.54)	3.63 (1.32-8.49)	0.20	0.41	0.08
miR-21 (RQ, median, range)	0.77 (0.3-1.9)	0.65 (0.31-1.98)	0.71 (0.32-1.85)	0.82 (0.5-2.4)	0.83 (0.53-2.31)	0.66 (0.34-1.41)	0.39	0.15	0.75
miR-30d (RQ, median, range)	0.81 (0.2-2.55)	0.72 (0.35-1.85)	0.76 (0.37-1.28)	0.68 (0.29-2.27)	0.99 (0.4-1.84)	0.61 (1.78-1.65)	0.56	0.9	0.14
miR-122 (RQ, median, range)	0.45 (0.08-3.60)	0.33 (0.10-2.61)	0.43 (0.10-2.07)	0.69 (0.06-2.48)	0.84 (0.10-2.18)	0.44 (0.20-5.74)	0.03	0.12	0.53
miR-133a (RQ, median, range)	0.34 (0.01-4.52)	0.46 (0.01-15.45)	0.64 (0.01-3.75)	1.28 (0.01-3.79)	0.29 (0.01-2.42)	0.30 (0.01-2.75)	0.5	0.49	0.16
miR-210 (RQ, median, range)	0.57 (0.17-5.12)	0.85 (0.05-4.05)	0.61 (0.13-3.08)	0.79 (0.03-3.61)	0.63 (0.02-3.59)	0.78 (0.04-4.01)	0.85	0.85	0.81
miR-486 (RQ, median, range)	0.87 (0.17-3.14)	0.69 (0.32-3.15)	0.56 (0.33-1.59)	0.73 (0.21-2.48)	0.93 (0.36-1.89)	0.60 (0.17-1.01)	0.51	**0.05**	0.63

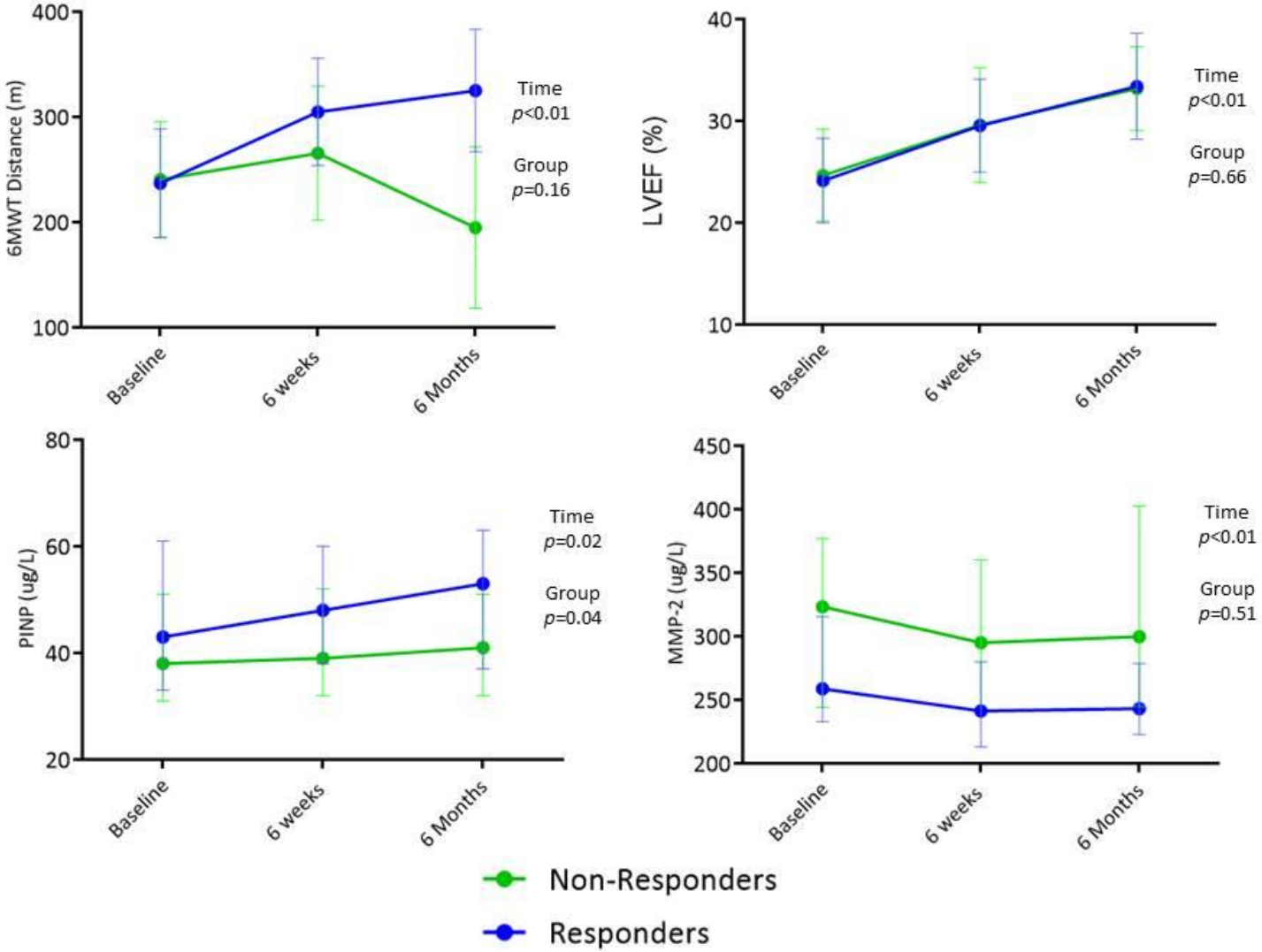


Figure 6.2 Trends in functional variables, LV geometry and biomarker expression following CRT implantation in responders and non-responders. Trends represent the mean value of responders or non-responders. Differences over time and between response status tested

6.4.4 Correlation between change in biomarker expression and cardiovascular variables following CRT

Correlation analyses of relative change (follow-up - baseline/baseline) over the short and long-term were undertaken between pre-specified biomarkers and functional, echocardiographic and NT-*pro*-BNP parameters. **Figure 6.3** demonstrates the strongest associations demonstrated in the exploration of the relationship between changes in parameters following CRT. Further significant associations in short term relative changes following CRT implantation were observed between LVESV/PIIINP (r=0.39, *p*=0.04) and NT-pro-BNP/miR-133a (r=-0.34, *p*=0.03). Moreover, additional associations were also observed between relative long-term changes in NT-*pro*-BNP with miR-133a (r=-0.50, *p*=<0.01) and MMP-9 (r=-0.32, *p*=0<0.03).

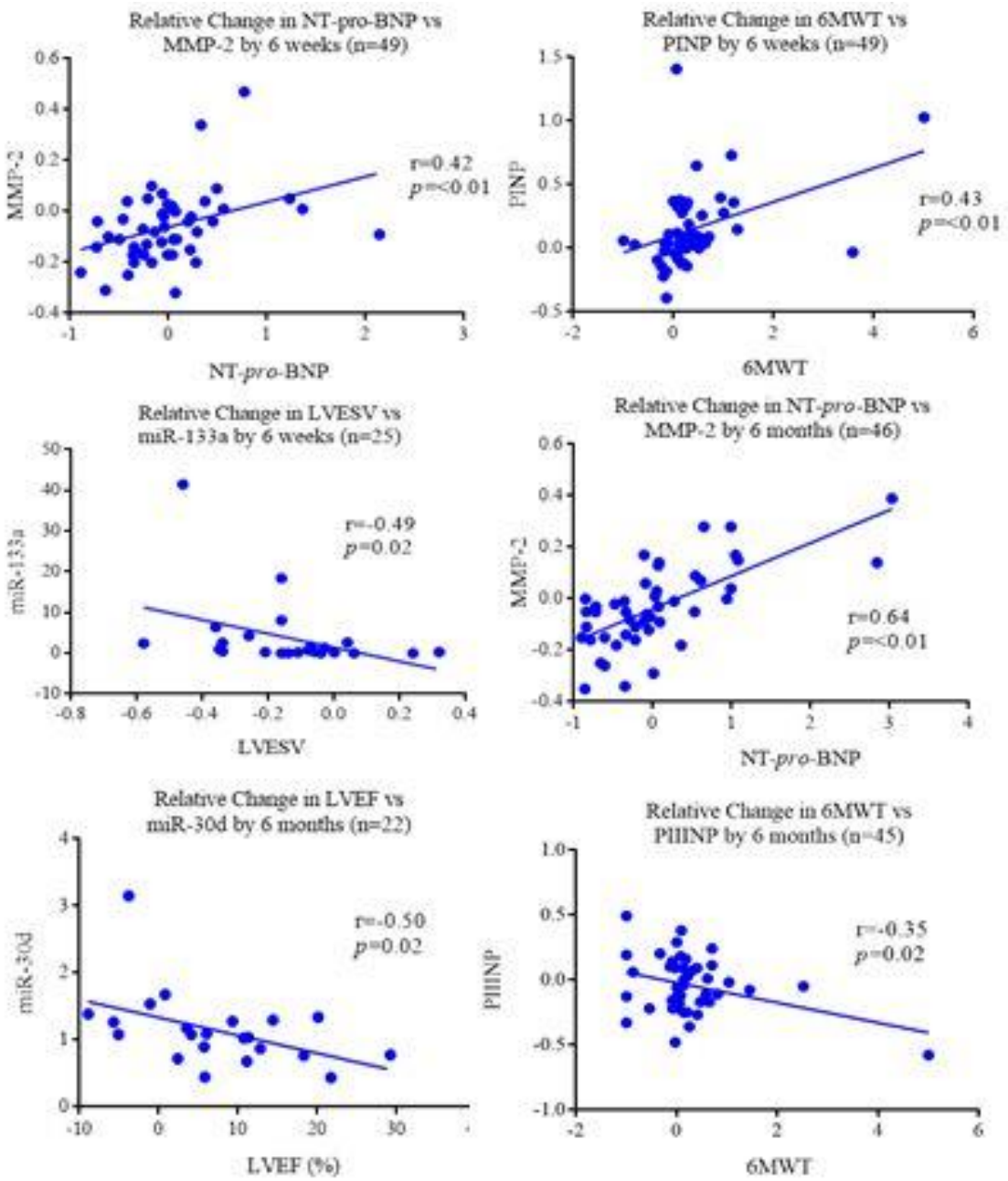
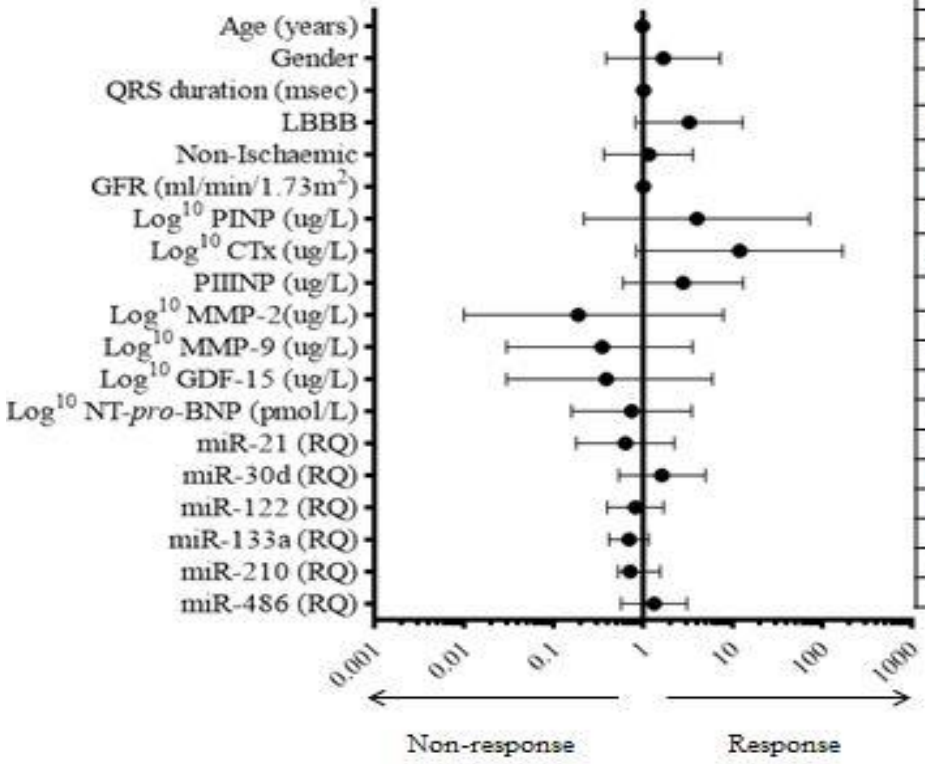


Figure 6.3 Bivariate correlation analyses of short and long-term changes following CRT between biomarkers versus functional and echocardiographic variables. Relative change applied to short (6 weeks) and long-term (6 months) reviews compared to the baseline assessments. Relative change was calculated by follow-up-Baseline/Baseline. Parametric or non-parametric bivariate correlation analysis performed dependent of continuous data distribution. Specific variables all pre-specified biomarkers compared to were 6MWT, QoL score, NT-*pro*-BNP, LVESV and LVEF.

6.4.5 Predicting Functional Response

Pre-specified baseline ECM, GDF-15 and miRNA biomarkers, alongside established clinical parameters underwent logistic regression modelling to build a prediction model for functional response. **Figure 6.4** demonstrates the univariate and multivariate model for pre-implant parameters ability to predict functional response. There were two variables on multivariate modelling trended towards being able to predict long-term functional response; increasing baseline CTx expression and presence of LBBB morphology on resting 12 lead ECG.



Univariate Regression			Multivariate Regression		
OR	P-Valve	CI (95%)	OR	P-Valve	CI (95%)
0.99	0.74	0.93-1.05			
1.69	0.48	0.39-7.26			
1.02	0.11	0.98-1.05			
3.29	0.09	0.83-12.95	3.53	0.09	0.84-14.80
1.16	0.8	0.37-3.61			
1.01	0.31	0.99-1.04			
4.02	0.35	0.22-72.79			
12.04	0.07	0.85-170.8	13.63	0.06	0.87-213.78
2.8	0.19	0.60-13.1			
0.19	0.39	0.01-7.92			
0.35	0.38	0.03-3.60			
0.39	0.5	0.03-5.94			
0.75	0.72	0.16-3.54			
0.64	0.49	0.18-2.25			
1.63	0.39	0.54-5.0			
0.83	0.61	0.40-1.71			
0.7	0.18	0.42-1.17			
0.72	0.91	0.53-1.56			
1.33	0.52	0.57-3.11			

Figure 6.4 Univariate and Multivariate Regression Model of Pre-CRT implant variables for prediction of functional response at 6 months. Forrest plot demonstrated the odds ratio and 95% confidence interval for parameters in univariate analysis. The table demonstrated the final step in the multivariate analysis. ECM, GDF-15 and NT-pro-BNP were logarithmically transformed for the prediction model.

6.4.6 Echocardiographic response and baseline biomarker expression

Echocardiographic response was established in 28 participants, due to limitations in the ability to perform paired LV volumetric assessments due to image quality, body habitus and ability to have a scan. There were 12 (42.9%) responders and 16 (57.1%) non-responders who did not. Baseline comparison of biomarker expression was performed between these echocardiographic responders and non-responders (**Table 6.4**). MMP-2 baseline expression was higher in those that did not reverse remodel enough to be defined a responder.

Table 6.4 Comparison of Baseline Biomarker Expression between with and without >15% Reduction in LVESV at 6 Months Following CRT Implantation.

Biomarkers	**Responders = 12**	**Non-Responders = 16**	**p-value**
PINP (ug/L, median, range)	39.5 (24.0-69.0)	48.0 (26.0-141.0)	0.14
CTx (ug/L, , median, range)	0.47 (0.14-1.14)	0.48 (0.18-0.90)	0.94
PIIINP (ug/L,mean±SD)	1.05±0.43	1.08±0.46	0.87
MMP-2(ug/L,median, range)	247.5 (155.3-671.5)	342.5 (194.0-789.5)	0.05
MMP-9 (ug/L, median, range)	77.5 (24.7-182.3)	70.4 (13.6-204.2)	0.51
GDF-15 (ug/L, median, range)	1.96 (1.12-4.28)	2.46 (1.20-10.29)	0.24
miR-21 (RQ, median, range)	0.86 (0.50-1.40)	0.77 (0.60-2.30)	0.63
miR-30d (RQ, median, range)	0.64 (0.34-1.68)	0.91 (0.35-1.86)	0.39
miR-122 (RQ, median, range)	0.46 (0.06-3.28)	0.84 (0.09-3.60)	0.16
miR-133a (RQ, median, range)	0.53 (0.01-3.79)	0.80 (0.08-2.26)	0.66
miR-210 (RQ, median, range)	0.42 (0.17-2.05)	0.85 (0.06-5.12)	0.15
miR-486 (RQ, median, range)	0.81 (0.17-1.93)	0.72 (0.21-2.48)	0.98

6.4.7 MACE and Baseline Biomarker Expression

During the observation one year period following CRT implantation therewere 8 (15.4%) MACE's at a median of 2.8 (0.1-11.9) months. There were 4 (7.7%) all-cause mortality events at a median of 3.7 (0.1-9.6) months. There were 6 (11.5%) hospital admissions for first heart failure event after CRT implantation which occurred at a median of 2.8 (1.3-11.9) months. Baseline characteristics for those with and without a MACE were not significantly different for any parameter. **Table 6.5** compares baseline biomarker expression dependent on MACE occurrence in the following year.

Table 6.5 Comparison of Baseline Biomarker Expression for Participants with and without MACE at 12 Months Following CRT Implantation.

Biomarkers	MACE = 8	No MACE = 44	p-value
PINP (ug/L, median, range)	50.0 (17.0-113.0)	40.0 (15.0-141.0)	0.36
CTx (ug/L, median, range)	0.43 (0.29-0.56)	0.38 (0.14-1.14)	0.41
PIIINP (ug/L,mean±SD)	1.25±0.49	1.01±0.37	0.15
MMP-2(ug/L, median, range)	332.3 (164.2-434.5)	281.9 (155.3-789.5)	0.63
MMP-9 (ug/L, median, range)	82.3 (45.1-145.5)	70.7 (13.6-254.1)	0.27
GDF-15 (ug/L, median, range)	2.82 (1.2-4.55)	2.64 (1.05-10.29)	0.76
miR-21 (RQ, median, range)	0.79 (0.50-1.0)	0.75 (0.30-2.40)	0.86
miR-30d (RQ, median, range)	0.53 (0.35-0.87)	0.71 (0.20-2.55)	0.05
miR-122 (RQ, median, range)	0.60 (0.14-1.38)	0.50 (0.06-3.60)	0.54
miR-133a (RQ, median, range)	1.23 (0.02-3.35)	0.53 (0.01-4.52)	0.47
miR-210 (RQ, median, range)	0.79 (0.06-1.43)	0.68 (0.17-5.12)	0.61
miR-486 (RQ, median, range)	0.77 (0.28-1.90)	0.76 (0.17-3.14)	0.57

6.4.8 Coronary Sinus Biomarker Profile

Coronary sinus and peripheral venous sampling was conducted on a subset of the COVERT-HF study cohort (n=26) on the day of CRT implant (only paired samples were compared). Haemolysed samples were excluded from the respective analyses. **Figure 6.5** shows those biomarkers that demonstrated significant variation in their expression. Expression was higher in the coronary sinus for hs-TnT ($p<0.01$) miR-30d (fold change 1.29, $p=0.05$) and -133a (fold change 3.36, $p<0.01$). Notably miR-486 had significantly higher expression in the coronary sinus (fold change 1.43, $p<0.01$), which is in keeping with the higher haemolysis rate observed in the coronary sinus sampling. PINP, PIIINP and MMP-2 all had significantly higher expression in peripheral samples than in the coronary sinus ($p<0.01$), this was observed for both responders and non-responders ($p<0.01$).The hs-TnT demonstrated significantly higher expression in non-responders in the coronary sinus than peripheral samples (28.8 ng/L (6.5-61.6) vs 38.4 ng/L (10.3-60.6), $p<0.01$) No statistical difference in hs-TnT levels for responders was observed between peripheral and coronary sinus samples (23.4 ng/L (8.5-68.2) vs 27.5 ng/L (17.7-64.7), $p=0.14$).

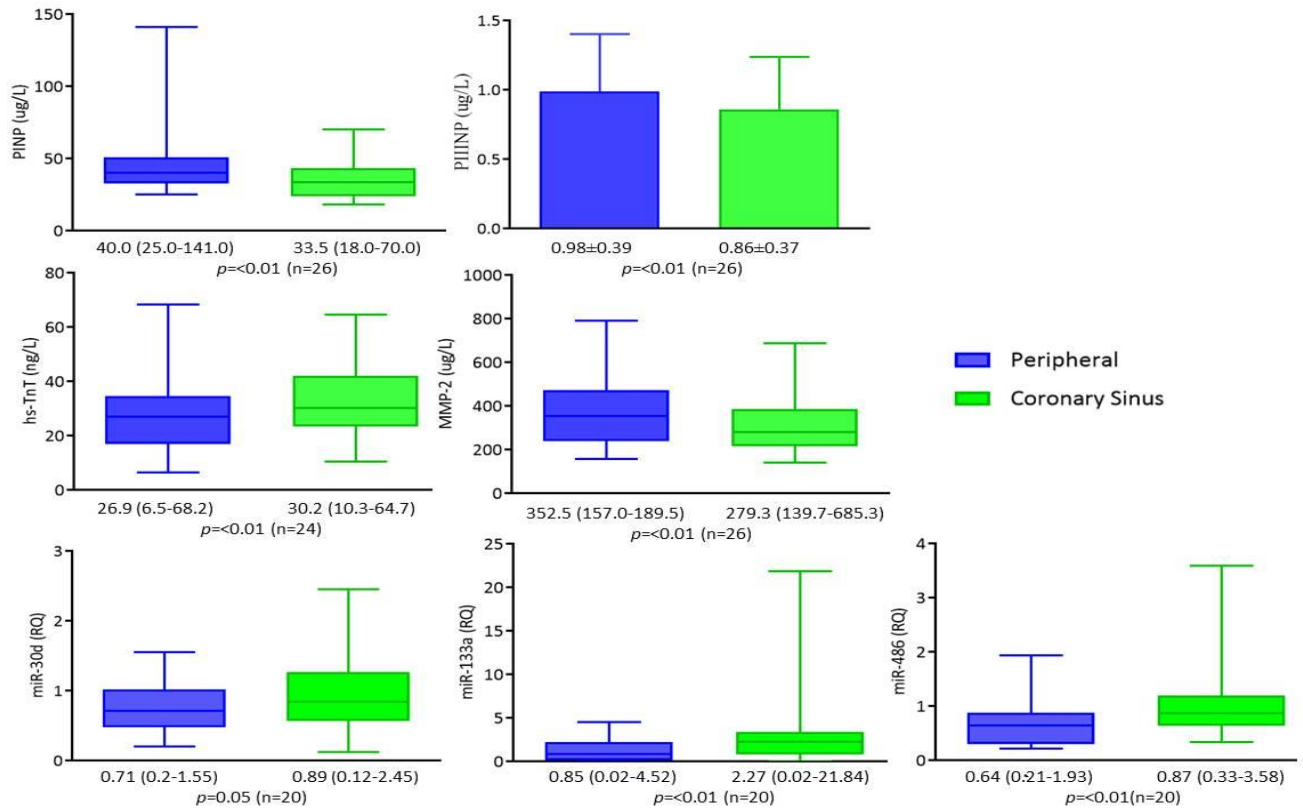


Figure 6.5 Variation between biomarker expression in peripheral and coronary sinus blood. PIIINP is expressed as mean±SD and underwent parametric comparison.PINP, MMP-2, hs-TnT, miR-30d, miR-133a and miR-486 were reported as median (range), and underwent non-parametric comparison. Comparisons were undertaken on the number of paired datasets available (n given for each comparison).

6.5 **DISCUSSION**

The COVERT-HF study is the first, prospective observational study to examine cardiac fibrosis biomarkers and dysregulated miRNA in HF patients undergoing CRT as to determine their potential to predict functional response. It is the first study, to our knowledge, to profile specific miRNAs that are known to be dysregulated in HFrEF patients. Selected baseline biomarkers and clinical variables did not demonstrate the ability to predict functional response, although CTx and LBBB morphology trended towards significance. However, levels of PINP and miR-122 following CRT implantation were shown to vary significantly between responders and non-responders. Expression over time in both groups was shown to alter for MMP-2,-9 and hs-TnT. Furthermore, specific changes in biomarker expression have shown to be associated with changes in functional, neurohormonal and LV geometry parameters both in short and long-term follow-up. The study also observed higher expression of cardiac fibrosis biomarkers systemically and higher expression of miRNAs in the CS.

Cardiac fibrosis biomarkers are known to be strongly associated with poor HF outcomes.[94,123,136] Alteration in cardiac ECM turnover is a key feature of cardiac fibrosis and is strongly associated with the development and progression of HFrEF.[113,134,136] Over the last decade ECM biomarkers have been associated with poor HF outcomes[134,136] and have shown the ablity to predict response to CRT.[127] Alteration in collagen synthesis and deposition, demonstrated by PINP/PICP for type I and PIIINP for type III have been observed to predict response in several observational studies for both functional and echocardiographic criteria. The exact behaviour and significance of collagen turnover has not been consistently replicated. [119,126,127,244] This inconsistency is principally due to the

variation in response definitions used. No synthesis, deposition or degradation biomarkers observed in our observational study demonstrated the ability to predict response. CTx trended towards the ability to predict response in a multivariable model, but it was not significant. No statistically significant differences in ECM biomarker expression was demonstrated between echocardiographic responders or those participants with or without a MACE. The CARE-HF trial[23] sub-study was the largest (n=260) to examine ECM biomarkers (PINP, PIIINP, ICTP, MMP-1) as potential predictors of response (survival and LVEF >35% at 18 months) and their findings observed that none of the biomarkers could predict response, supporting our observations.[125] Short-term changes in PINP, MMP-2 and PIIINP expression have been observed to correlate with 6MWT, NT-*pro*-BNP and LVESV changes respectively. Moreover, long-term changes in MMP-2 and PIIINP have been shown to correlate with NT-*pro*-BNP and 6WMT changes respectively. However, PINP in the short-term had a direct correlation with change in 6MWT distance, whereas PIIINP has a negative correlation with 6MWT distance change over the long-term, suggesting an association between collagen type I and III and functional response, which changes with time. These results imply greater increase in PINP expression correlates with the ability to increase the 6MWT test distance following CRT implantation, which reflects greater ECM turnover. This observation is made by Garcia-Balao *et al* in their observation study over 12 months.[126] Our observations however, demonstrate that a reduction in PIIINP expression at 6 months is associated with a greater 6MWT distance from baseline, suggesting reversal of cardiac fibrosis is associated with an improved functional status. PIIINP is observed to be more sensitive to changes in cardiac modelling, meaning this may represent a more long-term remodelling pattern. However in the long-term these markers do not correlate with LV geometry parameters, therefore it remains unclear the precise relationship between collagen turnover and

response to CRT. The ECM behaviour observations from the COVERT-HF study and the wider literature represent a heterogeneous response, which is likely to relate to the variety of factors causing the geometric changes seen in HFrEF.

Interestingly it is observed that PINP, PIIINP and MMP-2 expression are detected at higher levels in peripherally sampled blood compared to samples from the CS. These observations are in line with previous findings of Tolsana *et al*,[294] reporting that MMP-2 was more highly expressed systemically than in the CS. Multiple difference cell types have been demonstrated to secrete pro-MMP-2 that are not exclusive to the heart.[116] The implications of these particular observations are that HF modifies systemic expression of MMP-2 alongside that of the heart. Furthermore, greater expression is seen systematically, exerting that potentially modification of cardiac ECM in HF is contributed to by non-cardiac sources.

GDF-15 is a marker of myocardial stress and its ability to predict poor HF outcomes is well described.[110] Previously GDF-15 has been demonstrated to be a robust predictor of mortality following CRT implantation.[112] However, GDF-15 did not demonstrate the ability to predict functional response (survival & no HF hospitalisations, ↓≥1 NYHA class or ↑≥25% 6MWD at 1 year).[112] Our observations support these conclusions that GDF-15 cannot predict response, however we did not observe any difference in baseline expression in those participants with and without MACE.

There is maladaptation of complex cardiovascular biological systems in HF. This maladaptation involves dysregulation of specific miRNAs which regulate and control these systems.[139,148] MiRNA dysregulation has been demonstrated recently to occur and be

associated with the development of the adverse cardiac remodelling in HFrEF.[139] Marfella *et al*[197] were the first to observe altered expression in miRNA profiles between responders and non-responders using a miRNA microarray. Melman *et al*[199] more recently in a validation and translational study identified miR-30d was overexpressed in responders and had the ability to predict response status (increase LVEF >10% at 6 months). Neither study replicated the results of the other, however the studies significantly differed in the methodology employed. Marfella *et al*[197] and Melman *et al*[199] both used small cohorts with different characteristics, and different quantification methods (microarray and quantitative PCR respectively). In our study we selected six specific miRNAs that have been demonstrated to be dysregulated in HFrEF including miR-30d. MiRNAs were individually profiled by quantitative PCR methods, which is the gold standard for quantification.[310] None of the miRNAs were observed to be predictors of functional response and no variation at baseline was observed for echocardiographic responders. However, it was observed that miR-30d had statistically higher expression in patients with no MACE at 12 months. This observation has not previously been made, and implies increased LV wall stress is protective; however, this observation has not been tested in a prediction model against other variables. Over the observation period miR-122 expression was found to be statistically lower in functional responders. Recently miR-122 has been shown to be expressed in the liver due to congestion, which would support the observation there is lower expression in responders after CRT implantation.[311] Higher expression of miR-30d and -133a was observed in the coronary sinus compared to the peripheral circulation. These observations replicate previous findings that demonstrated these miRNAs to be enriched.[181] MiR-486 demonstrated higher expression in the coronary sinus than peripherally, potentially reflecting the higher haemolysis rate of samples taken via the catheter.

The overall cohort behaved as expected following CRT implantation.[22,23,31,126,294] Overall, the patients had a significantly improved NYHA classification and 6MWT distances alongside reducing QoL scores. Interestingly the entire cohort showed a statistically significant improvement in LV geometry and reduction in hs-TnT over the observation period, without any difference between responders and non-responders being observed. The pattern observed in overall improvement in LV geometry has been well described following CRT implantation[23,31] but the pattern not being replicated in functional responders only emphasises the known poor correlation between echocardiographic and clinical response criteria.[1] Variation in different definitions used for response remains a major limitation of research in this field.[1]

6.5.1 Study Limitations

There are several limitations to our prospective study that should be reflected upon during the interpretation of our results. Firstly, this was a small single-centre proof-of-concept study. Secondly, transthoracic echocardiography was limited in several participants due to body habitus and illness, resulting in the inability to perform paired standardised modified Simpson's biplane assessments on all scans. Thirdly, the NICE 2014[17] guidance was released during the trial period reflecting a change in the guidelines moving away from mechanical dys-synchrony on echocardiography and towards QRS duration and morphology. The study inclusion changed to reflect the real world circumstances of the study, but this may have altered the cohort characteristics. Fourthly, the numbers of MACE events was low in the 12 months observed and results must be interpreted cautiously. Finally, coronary sinus samples were taken on the second half of the cohort following permission to perform the assessment being granted. This was a logistical limitation of the study and formed a practical

problem of extrapolating the implication of the results from such small numbers, in what formed essentially a sub-study. Continous sampling of CS samples was performed on the second half of the cohort; however the total numbers of participants in the sub-study was low. Caution must be applied when interpreting these specific results.

6.6 CONCLUSION

In conclusion, our proof-of-study, did not demonstrate that ECM biomarkers, GDF-15 or specific miRNAs can predict functional response in a heterogeneous HFrEF patient population undergoing CRT. We observed LBBB morphology and the biomarker CTx did show a trend towards predicting response and warrant further study.

6.7 PUBLICATIONS

This chapter formed the basis of a poster presentation at the Sankey Cliinical Competion (University Hospitals Birmingham) which won best poster presentation. The paper has been recently submitted for consideration for publication with Heart Rhythm at the time of submission of this thesis. .

Chapter Seven

BODY COMPOSITION IN HEART FAILURE AND IMPACT OF CARDIAC RESYNCHRONISATION THERAPY

7.1 INTRODUCTION

There is a complex relationship between HF, body composition and metabolism with the interplay altering with the development and progression of HF.[218] The development of HF causes neurohormonal activation, a pro-inflammatory state and endothelial dysfunction.[206,207] The degree of metabolic systems shifting into a pro-catabolic state in HF is heavily influenced by the patient's body composition.[218] Presence of obesity makes the development of HF more likely,[212] but the presence of higher adiposity is protective.[213] This counterintuitive observation is referred to as the '*obesity paradox*'.[212,214] Higher adiposity is inversely related to neurohormonal activation and is observed to be protective against progression of HF.[208,209]

More complex relationships between different body composition components and HF have been observed. Sarcopenia is associated more with HFrEF and a more pro-inflammatory state[211] and neurohormonal signalling.[209] CC reflects the body composition of the pro-catabolic state seen in HF and is an important and reproducible variable in predicting poor HF outcomes.[207,212,213] All body components have been shown to be affected by CC and regulated by elevated neurohormonal and inflammatory signals.[207,208] The presence of CC represents a critical step in the pro-catabolic transition of the body's metabolism and body composition in advancing HF.[218] Our own detailed peer-reviewed article summarises the literature on the complex interplay between HF, BC and metabolism, highlighting the importance on outcomes, but also the very limited evidence currently.[218]

CRT implantation provides a unique opportunity in the study of HFrEF to see how reverse remodelling and improvement in the patient alters the patient's body composition. Cai *et al*[216] observed in a retrospective cohort study of 219 Chinese HFrEF (LVEF<35%) that overweight (24.0-28.0 kg/m^2) and obese (>28.0 kg/m$^{2)}$ BMI predicted response to CRT and improved survival at 6 months. This observation represents the potential importance of body composition in predicting response to CRT, although there are limitations in drawing any significant conclusions from this study. Firstly it was based on a far eastern population and is not directly applicable to western populations.[216] Secondly, no account of specific body composition components was made or whether CC was present.[216] Thirdly, the study demonstrated obese population better tolerated OMT, which may have contributed to the improved response and outcomes.[216] This study is the only one study examining body composition and CRT. Treatment of HFrEF with CRT and the potential response including cardiac remodelling is an potential opportunity to examine impact on body composition component and whether there are any changes.

7.2 AIM AND OBJECTIVES (Chapter 2)

To examine the impact of body composition in patients with HFrEF and dys-synchrony undergoing CRT.

7.3 METHODOLOGY (Chapter 3.5)

A small physiological pilot as part of the COVERT-HF prospective observational study (sub-study) was undertaken on unselected HF patients undergoing CRT implantation meeting

NICE (TA120)[17] criteria between September 2014 and December 2015. In July 2014 a substantial amendment to the regional ethics committee approved performance of air-displacement plethysmography assessment. All participants recruited beyond this point were asked to participate in the sub-study as part of the informed consent process. Air displacement tomography was conducted at all three defined research visits (pre-implant, post-implant: 6 weeks and 6 months) alongside NYHA classification, 6MWT, echocardiography, MLHFQ, ECG and blood sampling. The methods employed in the COVERT-HF prospective cohort study are outlined in detail in **Chapter 5.5 and Chapter 6. 3**. This sub-study is the first to examine body compostion alterations following CRT implantation. It is a small sub-study, which may be hypothesis generating. The recruitment was a small number of participants and was not powered being a pilot study.

7.3.1 Device Implantation (Chapter 3.2)

Device implantation was undertaken as a day-case procedure according to local protocol.[312]

7.3.2 Transthoracic Echocardiography (Chapter 3.5)

All participants underwent transthoracic echocardiography (Vivid 7, GE Healthcare, Horten, Norway) examination for LV volumetric assessment. LV volumetric assessment according to the prescribed literature[201] by the same nationally accredited[200] operator on the same machines. All measurements were analysed offline (EchoPac, GE Healthcare, Horten, Norway).

7.3.3 Air-Displacement Plethysmography (Chapter 3.5)

Whole body air-displacement plethysmography (BOD POD® Life Measurement Inc, Concord, California, USA) reliably and reproducibly measures body composition.[261] The BOD POD® is comparable with more traditional methods of measuring body composition.[261] Participants were required to starve and not exercise two hours prior to the test. The participant had a height measurement performed and was weighed on the calibrated scales attached to the BOD POD®. All participants were asked to enter the BOD POD® wearing a lycra swim cap and their underwear only for standardisation of the measurements. Two serial measurements were performed for body composition and the average was taken as the result, where measurements between tests varied significantly the operator was asked to perform a third test.

7.3.4 Blood Sampling and Laboratory Analysis (Chapter 3.5)

Peripheral venous sampling of blood was performed following two hours of fasting and one hour of rest. Peripheral samples at implantation were taken the morning of the implant. Clinical laboratory measurements were performed according to standard hospital procedure including NT-*pro*-BNP.

7.3.5 Study Outcomes (Chapter 3.5)

The primary outcome measure for the study was the patients' functional response status. Functional responders were defined as those whom survived, did not undergo heart transplantation and achieved two out of three response criteria (↓≥1 NYHA, ↑≥10% 6MWT distance, ↓MLHFQ score>5) at 6 months follow-up

7.3.6 Statistical Analysis (Chapter 3.8)

Categorical variables were reported as frequency and percentages. Comparison analyses for categorical data were performed using the Chi-Squared or Fishers Exact tests dependent on appropriateness. Continuous data underwent histogram plots for assessment of normality. Normally distributed data were reported as mean ± SD and comparative analysis was performed using independent t-tests. Non-normally distributed data were reported as median (full range) and were compared using a Mann-Whitney U test. Variation in continuous variables over three time periods was analysed using either one-way analysis of variance (ANOVA) or Friedman test, respectively. Mixed between-within subjects analysis of variance was used to compare variation in body composition data in functional responders and non-responders over 6 months of observations. Bivariate correlation analyses (Pearson (parametric) or Spearman rank (non-parametric) estimators) was performed between change in body composition and functional, echocardiographic and neurohormonal parameters. A p-value <0.05 was considered statistically significant.

7.4 RESULTS

There were 27 participants enrolled in the sub-study, however only 25 were able to undertake baseline body composition assessment. One participant was excluded from the sub-study due to being unable to undertake assessment in the BOD POD®. The other participant had significant erroneous body composition baseline measurements despite repeated tests. These exclusions made no difference to sub-study cohort characteristics. **Figure 7.1** demonstrates the recruitment and flow of patients through the sub-group within the COVERT-HF study.

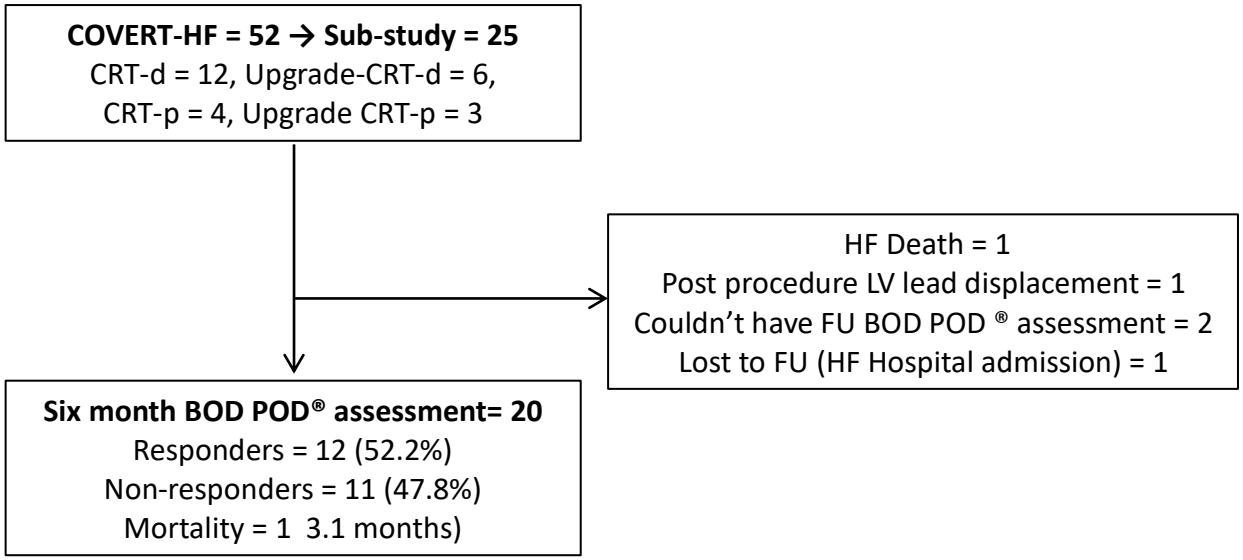


Figure 7.1 Recruitment and Flow of Participants through body composition sub-study.

7.4.1 Baseline Sub-Group Characteristics

The sub-groups characteristics are summarised in **table 7.1.** Functional responders and non-responders characteristics were compared within the sub-group. Response status at 6 months was determined in 23 participants with body composition measurements. The two others in the group could not have a 6 month response status performed (one post procedure exclusion and one lost to follow-up). Comparatively three participants whom did have a functional response status were unable to have a 6 month body composition assessment performed (1 death during follow-up and two not well enough to undergo assessment).The first follow-up occurred at a mean(±SD) 1.7±0.3 months and the final research visit was at 5.8±0.5 months.

Table 7.1 Baseline Characteristics

	Total Cohort =25	Responders=12	Non-Responders =11	p-value
Demographics				
Age (years, mean±SD)	73.4±10.0	68.1±14.4	76.0±7.4	0.19
Male (n,%)	23 (92.0%)	12 (100.0%)	9 (81.8%)	0.42
Device				
CRT-D (n,%)	18 (72.0%)	8 (66.7%)	9 (81.8%)	0.73
Upgrade (n,%)	9 (36.0%)	2 (16.7%)	7 (63.6%)	0.06
Aetiology				
Ischaemic (n,%)	16 (64.0%)	6 (50.0%)	8 (72.7%)	0.49
Non-ischaemic (n,%)	9 (36.0%)	6 (50.0%)	3 (27.3%)	
Co-morbidities				
Diabetes Mellitus (n,%)	6 (24.0%)	2 (16.7%)	4 (36.4%)	0.55
CKD (n,%)	13 (52.0%)	7 (58.3%)	6 (54.5%)	1.00
NYHA (n,%)II	10 (43.5%)	5 (41.7%)	5 (45.5%)	0.52
III	12 (52.2%)	7 (58.3%)	5 (45.5%)	
IV	1 (4.3%)	0 (0.0%)	1 (9.1%)	
Electrocardiogram				
AF (n,%)	9 (36.0%)	4 (33.3%)	4 (36.4%)	1.00
LBBB (n,%)	17 (68.0%)	9 (75.0%)	6 (54.5%)	0.56
QRS (msec, median, range)	162(120-212)	168 (138-212)	160 (138-194)	0.60
6MWT (M, mean±SD)	252.6±132.0	291.9±133.3	215.3±147.4	0.29
QOL Score (median, range)	48.0 (8.0-85.0)	55.5 (9.0-85.0)	29.0 (8.0-68.0)	0.11
Laboratory Tests				
eGFR (ml/min/1.73m^2, median,range)	52.0 (25.0-130.0)	52.5 (25.0-130.0)	52.0 (26.0-79.0)	0.61
NT-*pro*-BNP (pmol/L, median, range)	267.0 (75.0-4138.0)	237.0 (75.0-4138.0)	273.0 (133.0-547.0)	0.33
Medications				
ACEi/ARB (n,%)	25 (100.0%)	12 (100.0%)	11 (100.0%)	1.00
BB (n,%)	21 (84.0%)	10 (83.3%)	10 (90.9%)	0.62
MRA (n,%)	14 (56.0%)	7 (58.3%)	5 (45.5%)	0.38
Echocardiography≠				
LVESV (ml,median,range)	125.8 (62.9-268.7)	136.7(80.7-268.7)	110.9(62.9-169.4)	0.29
LVESV_BSA(ml,median,range)	58.9 (38.7-128.0)	66.1 (42.7-128.0)	59.1 (38.7-85.8)	0.35
LVEF (%, median,range)	25.6 (9.7-35.4)	24.4 (10.0-34.4)	28.6 (9.7-35.4)	0.40
Body Composition				
BMI (kg/m2, median, range)	28.7 (22.4-41.9)	29.3 (22.4-37.3)	27.8 (23.9-40.8)	0.85
Fat Mass (kg, median, range)~	31.4 (18.1-61.2)	31.3 (19.3-56.4)	29.7 (18.1-58.7)	0.81
Relative FM (median, range)~	0.38 (0.23-0.54)	0.35 (0.28-0.50)	0.42 (0.23-0.54)	0.29
Lean Mass (kg, median, range) ~	52.3 (30.9-73.3)	52.6 (40.3-73.3)	50.5 (30.9-62.5)	0.48
Waist circ (cm, median,range)~	98.2 (84.6-131.0)	97.5 (84.6-120.8)	98.2 (85.0-114.5)	0.79

≠ based on available data, FM = Fat Mass

Both responders and non-responders shared similar characteristics with no statistical differences observed. This observation included all the baseline body composition components. As expected there were no differences in patient symptoms, function, QoL and LV geometry characteristics over the 6 months of follow-up.

7.4.2 Effect of CRT on Cardiac Function and Body Composition

Table 7.2 demonstrates the impact of CRT over 6 months on body composition and cardiac function variables. One participant at 6 weeks and two participants at 6 months (including the same one at 6 weeks) could not/did not want to undertake the BOD POD® assessment; however they were able to complete the other assessments as part of the research visit. The data observed a significant improvement in MLHFQ scores and a decrease in QRS duration. Total and percentage fat mass demonstrated a trend towards a reduction 6 months after CRT implantation. There was also an expected trend in improvement in LVEF as is demonstrated in the wider COVERT-HF study. The clinical, functional, laboratory results in the sub-group correspond with those in the larger COVERT-HF study.

Table 7.2 Changes in Sub-Group Characteristics Over 6 Months

	Baseline	6 weeks	6 months	p-value
Clinical, Functional, LaboratoryΔ				
QOL Score (median,range)	48.0 (8.0-85.0)	33.5 (0.0-73.0)	23.5 (0.0-0.83)	0.06
QRS (msec, median,range)	162(120-212)	145 (102-194)	159 (112-214)	0.02
6MWT (M, mean±SD)	252.6±132.0	277.1±145.2	242.4±178.8	0.29
eGFR (ml/min/1.73m^2, median,range)	52.0(25.0-130.0)	47.0(24.0-105.0)	52.7(20.0-90.0)	0.34
NT-*pro*-BNP (pmol/L, median,range)	267.0 (75.0-4138.0)	236.5 (27.0-3848.0)	272.5 (15.0-1690.0)	0.87
Body Composition (median, range)				
BMI (kg/m2)	28.7 (22.4-41.9)	28.5 (22.0-34.4)	28.0 (22.4-35.7)	0.39
Fat Mass (kg)~	31.4 (18.1-61.2)	29.0 (18.9-61.2)	29.1 (18.6-50.3)	0.12
Relative Fat Mass ~	0.38 (0.23-0.54)	0.34 (0.25-0.51)	0.39 (0.28-0.52)	0.09
Lean Mass (kg)~	52.3 (30.9-73.3)	53.9 (31.9-75.0)	50.5 (31.9-75.0)	0.19
Waist circ (cm)~	98.2 (84.6-131.0)	99.3(76.0-119.4)	100.1(78.9-120.9)	0.53
Echocardiography≠				
LVESV (ml, median,range)	125.8 (62.9-268.7)	112.7 (52.8-210.8)	95.2 (57.8-315.6)	0.26
LVESV_BSA(ml,median,range)	58.9 (38.7-128.0)	56.8(28.2-118.7)	49.5(30.6-131.5)	0.26
LVEF (%, median,range)	25.6 (9.7-35.4)	29.4(13.6-43.6)	32.7(14.4-41.7)	0.14

Δ based on patients able to have clinical, functional and laboratory assessments (baseline=25, 6 weeks=24, 6 months=22), ~based on participants able/available to have BOD POD ® assessment (baseline=25, 6 weeks=23, 6 months=20), ≠based upon complete series of echocardiograms with biplane measurements

7.4.3. Effects of CRT on Body Composition by Response Status.

The changes over time in body composition for functional responders and non-responders were analysed. **Table 7.3** demonstrates the trends in the changes in the body composition components for 6 months after CRT implantation. There were no significant differences between responders and non-responders observed. Furthermore there were no statistically significant changes in body composition over the 6 month observation period. However, there was a trend that was observed where relative fat mass was higher at baseline for non-responders and had a large decrease after 6 weeks following CRT implantation, compared to responders (**Figure 7.2**). Following the 6 week observation period the relative fat mass proportion returned to similar baseline levels at 6 months for non-responders. The trend towards there being a significant change in fat mass over time was driven by the amount of decrease in fat mass for non-responders in the first 6 weeks following CRT. Responders were observed to have minimal variation over the three observation points and to have lower levels of relative fat mass than non-responders (**Figure 7.2**). There was a trend towards a difference between responders and non-responders over time for fat mass.

Table 7.3 Behaviour of Body Composition Following CRT Implantation. Interaction between responder status and over time analysed.

	Responders			Non-Responders			P-Value		
Body Composition (median, range)	**Baseline**	**6 weeks**	**6 months**	**Baseline**	**6 weeks**	**6 months**	**Response**	**Time**	**Interaction**
BMI (kg/m^2)	29.3 (22.4-37.3)	29.7 (22.0-37.4)	28.8 (22.4-35.0)	27.8 (23.9-40.8)	27.3 (23.9-35.9)	27.6 (23.8-35.7)	0.98	0.28	0.71
Fat Mass (kg)	31.4 (19.3-56.4)	29.5 (18.9-61.2)	30.3 (18.6-50.3)	29.7 (18.1-58.7)	27.6 (19.6-47.7)	29.1 (24.5-45.6)	0.74	0.11	0.68
Relative Fat Mass ~	0.35 (0.28-0.50)	0.34 (0.28-0.50)	0.36 (0.28-0.44)	0.42 (0.23-0.54)	0.35 (0.25-0.51)	0.40 (0.32-0.52)	0.18	0.13	0.47
Lean Mass (kg)~	52.6 (40.3-73.3)	51.7 (43.6-75.0)	51.2 (42.5-74.4)	50.5 (30.9-62.5)	53.9 (31.9-62.4)	48.4 (32.5-62.3)	0.25	0.25	0.48
Waist circumference (cm)~	97.5(84.6-120.8)	98.8(85.6-114.5)	99.3(84.2-113.8)	98.2(85.0-114.5)	96.7(76.0-119.4)	103.2(78.9-120.9)	0.55	0.96	0.33

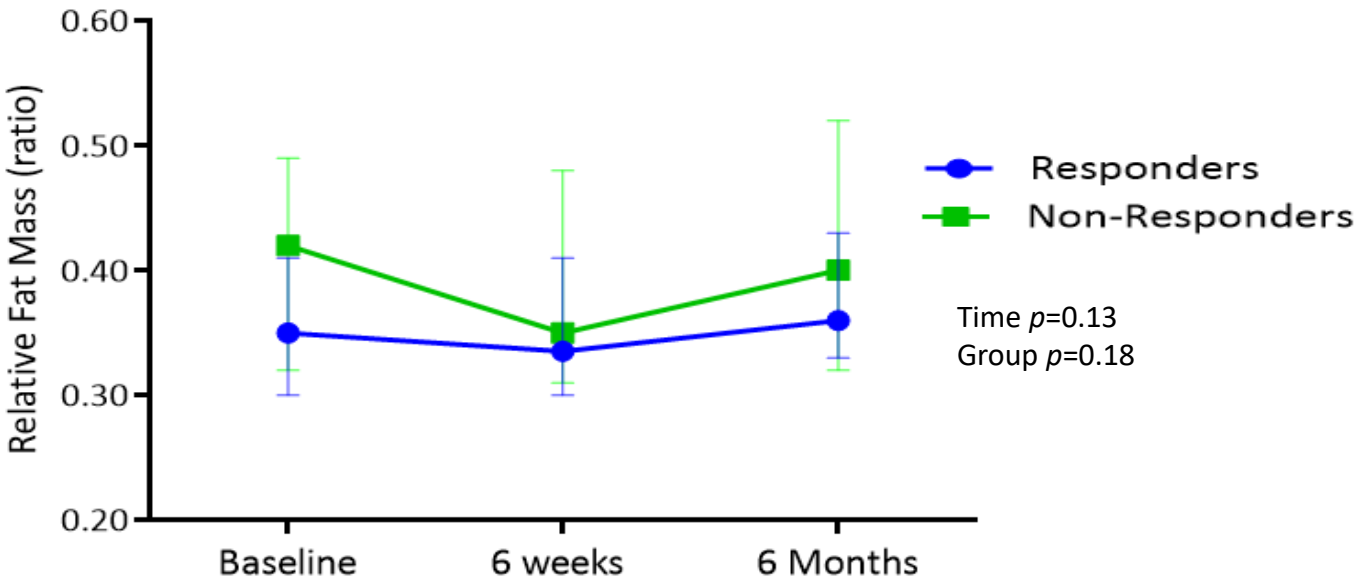


Figure 10.2 Change in Relative Fat Mass in Functional Responders and Non-Responders over 6 Months. . The median (CI 95%) is represented.

7.4.4 Correlations Analysis between Relative change in Body Composition and Cardiac Function

Bivariate correlation analysis for relative change (follow-up-baseline/baseline) in the short and long-term were undertaken between body composition components and cardiac function variables (functional, LV geometry and neurohormonal). **Figure 7.3** demonstrates the strongest associations demonstrated in the exploration of the relationship between body composition and cardiac function variables following CRT. A strong inverse correlation was demonstrated between LVESV volume index and relative fat mass (**Figure 7.3 A**). The strength of this association was reflected in the strength of the correlations between fat mass and LVESV (both measurements with and without volume indexing). A strong inverse correlation was observed between fat mass and NT-*pro*-BNP (**Figure 7.3 B**).There was an observed medium association between Fat Mass and eGFR (r=0.41, *p*=0.06) that trended towards significance at 6 weeks observation. Long-term the strongest inverse correlation was observed between fat mass and eGFR (**Figure 7.3 C**). A significant association was also observed between eGFR and lean mass (r=0.47, *p*=0.04) at 6 months after CRT implantation.

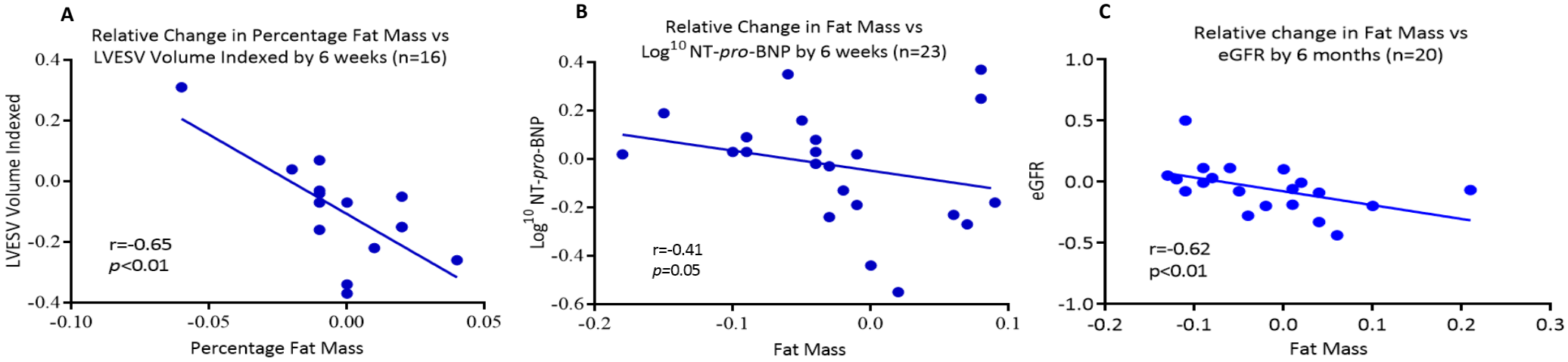


Figure 7.3 Bivariate correlation analysis of short and long-term changes following CRT between biomarkers versus functional and echocardiographic variables. Relative change applied to short (6 weeks) and long-term (6 months) research visits compared to the baseline assessments. Relative change was calculated by follow up-Baseline/Baseline. Parametric or non-parametric bivariate correlation analysis was performed dependent of continuous data distribution. All body composition components were compared in bivariate correlation analysis to MLHFQ, QRS duration, 6MWT, eGFR, NT-*pro*-BNP, LVESV, LVESV Volume indexed and LVEF. Fat Mass vs LVESV Volume indexed (**A**). Fat Mass vs Log^{10} NT-*pro*-BNP (**B**). Fat Mass vs eGFR (**C**).

7.5 DISCUSSION

This small physiological pilot is the first study as a proof-of-concept to examine body composition before and after CRT implantation, a sophisticated technology to resynchronise the dyssynchronous heart in advanced cardiac failure. This study examines the differences between patients who are functional responders and non-responders. We observed that there was an association between changes in fat mass and LV geometry shortly after CRT placement. Relative short-term changes in LVESV and LVESV volume indexed measurements were strongly inversely correlated with fat mass and relative fat mass over the first 6 weeks of observation. The long term relative change correlations however did not reflect the strength of the short term associations. There was no difference in body composition at baseline between responder and non-responders. The entire cohort demonstrated no statistical change in body composition components over 6 months, however there was a trend towards statistical significance for change over time for lean mass, fat mass and relative fat mass. Lean mass was observed to decrease following CRT implant. Fat mass and relative fat mass were observed to have a large decrease in levels at the first follow-up visit and then return to similar baseline levels at six months. This trend was observed to be driven by non-responders primarily. Importantly air-displacement plethysmography interprets body water as fat mass; therefore this will potentially exert an influence on the changes in body compostion as fluid retention improves following CRT. Moreover, there is no data examining the reporting of the reliability of air-displacement plethysmography in HF patients,[261] meaning the exact impact of body water is not clear. Finally renal function, specifically eGFR was found to be significantly inversely correlated with relative change in

fat mass at 6 months. Moreover this association was trending towards significance at the initial follow-up assessment at 6 weeks post implant.

The observed association between LV geometry and fat mass relative change following CRT implantation has not been observed previously. The relationship potentially reflects that increased fat mass is associated with reverse cardiac remodelling, in other words if fat mass is lost following CRT implantation, this is associated with progression in LV dilation. The mechanism of this potential relationship is not demonstrated in this study. The neurohormonal system would appear to be implicated though given the well described reduction in circulating natriuretic peptides in the presence of increased adiposity.[206,218] Adipocytes are sensitive to natriuretic peptides, activating lipolysis and enhancing expression of brown adipocyte genes, increasing thermogenesis, favouring the metabolic shift towards a pro-catabolic state in progressive HF.[206] Naturetic peptides critical role also known to stimulate the release of adipokines (specifically adiponectin and leptin) which increase energy utilisation and weight reduction.[206] The comparison of NT-*pro*-BNP and fat mass demonstrated a moderate strength inverse correlation; reflecting increasing levels of NT-*pro*-BNP immediately after CRT implantation is associated with decreasing levels of fat mass. The association is not reproduced when comparing relative change between pre-implant and 6 months follow-up measures. This observation supports the inverse association between natriuretic peptides and BMI/fat mass that has previously been described in the literature.[206,209] The observed correlations reflect those seen between fat mass and LV geometry. The observations are only seen over a short period post CRT implantation. There is also no significant association seen between changes in free fat mass

and LV geometry and neurohormonal variables. Suggesting that fat mass is intertwined with LV dysfunction and the neurohormonal systems and this is affected by the implantation of a CRT device. There is extensive evidence of the interplay between body composition, metabolism and HF and the critical role that adiposity plays in regulation of the pro-catabolic state. Higher levels of adiposity are protective from poor outcomes in HF, slowing progressive towards a pro-catabolic state. The precise mechanisms involved are not entirely clear with the limited evidence in this area. The impact of adiposity on the neurohormonal system reduces the circulating levels of natriuretic peptides and their ability to drive the metabolic shift in HF. The interplay between metabolism, bidy composition and HF is far more complex and interwined than the natruteic peptide system.[218] Fat mass specifically may influence the CRTs ability to induce reverse cardiac remodelling. This is a hypothesis that requires further investigation.

Limited evidence is available on the impact of CRT on body composition. The only study indirectly measuring of body composition was a retrospective analysis (n=219) of Chinese patients with severe LV systolic dysfunction with a wide spread of BMIs undergoing CRT implantation.[216] This study focused on initial BMI predicting response and cardiovascular outcomes at 6 months.[216] Cai *et al*[216] observed that overweight (24-28 kg/m^2) and obese (>28 kg/m^2) patients before implant had better responses and improved cardiovascular outcomes. There are limitations around drawing major conclusions on body composition and CRT from this study; firstly is it is based purely on a Chinese population where obesity is defined at different levels to Western population's, therefore the results are not particularly applicable to our population in the UK. Secondly, the study also observed that obese

patients better tolerated OMT, than non-obese patients and this may be partially the explanation for why these patients had better outcomes. Obesity has been observed to contribute to maintaining systolic blood pressure and preserving renal function, which physiologically explains why obese patients better tolerate OMT.[212] Thirdly, this study does not account for specific body composition components and uses BMI which is a very crude measure of body composition and cannot comment on the relative contribution of fat or lean mass.[212] Given these limitations and the potential impact of body composition on the success of CRT further study is required, especially into the specific role of fat mass and metabolic shifts in HF.

Deterioration in renal function and the presence of CKD stage 3 (eGFR <60ml/min/1.73m^2) and above is consistently and reproducibly finding that predicts non-response and poor cardiovascular outcomes following CRT implantation.[237,238,300] Our own retrospective study of all CRT implants 2009-2013 observed that CKD stage 3 and above was observed to be the strongest predictor of MACE and all-cause mortality in a multivariate prediction model (**chapter 7**). Importantly the prospective study demonstrated that eGFR when treated as a continuous variable in a multivariable logistic regression did not predict function response. Mechanistically progressive deterioration in eGFR is linked to progressive adverse cardiac remodelling.[302] The observations of the inverse correlation between eGFR and fat mass relative change during the 6 months observations suggests increasing fat mass is associated with deteriorating renal function. This is a paradoxical observation as obesity is thought to preserve renal function.[212] This evidence is related to BMI and does not account for fat mass exclusively. There was no association described between lean mass and BMI in this study.

Caution is advised in interpreting these results as eGFR is calculated using the modification of diet in renal disease equation[239] which utilises body surface area as a parameter. This means eGFR is not a completely independent variable and this may be the reason for the findings observed.

The sub-groups were very similar to the COVERT-HF cohort characteristics. The baseline characteristics were marginally different, with the subgroup having slightly more men, less ischaemic aetiology and LBBB. The clinical symptoms, 6MWT, MLHFQ and LV geometry variables did not vary at baseline between the COVERT-HF cohort and this sub-study. Proportionally the sub-group had similar levels of responders and non-responders to the larger cohort. The trends in the entire cohort over the three observation points, across six months demonstrated a significant decrease in MLHFQ and QRS duration. The significance of the QRS duration decrease is driven principally by the initial decrease from pre-implant to the 6 week observation point. The 6MWT did not show a significant change during follow-up, which initially might be expected. It however is an important discriminator between responders and non-responders. When responders and non-responders 6MWT are examined separately over 6 months the non-responders demonstrate no significant change in 6MWT (p=0.16) and the responders demonstrate a significant increase in 6MWT distance over 6 months (p<0.01), reflecting behaviour observed in the COVERT-HF cohort.

In the sub-study only observations on the overall trends for responders and non-responders could be made due to the small numbers of participants. This has allowed for hypothesis

generation. The next step in analysis would be to stratify patients by percentage fat mass and examine their behaviour and outcomes following CRT implantation. An additional consideration in further studies that could not be performed in this study was to catergorise patients by the presence of CC and see how a CRT implantation affects outcomes in these patients. The literature suggests this is a particularly critical step in the switching of body metabolism to a pro-catabolic state and infers particularly poor cardiovascular outcomes.[207,213,214] Both lean and fat mass are implicated in CC and examining these following CRT implantation would be a particularly important next step.

7.5.1 Study Limitations

This prospective study has several limitations that should be accounted for when interpreting these results. Firstly it is a sub-group analysis on a small number of participants from a single centre prospective cohort study, therefore conclusion generation will be limited. However, the study is hypothesis generating. Secondly, two participants completed research assessments, but were unable to have a BOD POD® assessment, both being graded as non-responders. This produces a participant selection bias. Thirdly, air-displacement plethysmography does not account for body water and measures it as fat mass, which means dependent on how oedematous the patient was, the results may be inaccurate. This potential bias was minimised as participants were recruited when well and not in decompensated HF (**Appendix Q**). Fourthly, though air-displacement tomography is validated in healthy populations, it has not yet been validated in HF patients. This poses a limitation in the accurancy of the body compensation measurments taken. The next step in body composition in HF patients using this mrthodology is a validation study. Fifthly, the

study was unable to account for the presence of CC, which has been demonstrated to be a critical variable in indicating advancing HF.[218] Finally not all patients undergoing BOD POD® were able to have a complete echocardiogram performed, limiting the ability to perform volumetric assessment of the LV. An important reason for this was body habitus, with participants being more obsess generally being the more challenging to perform a full echocardiogram. This issue offers an obvious reporting bias in the study.

7.6 CONCLUSION

This is the first prospective study to examine body composition components before and after CRT implantation. The observations suggest there is an overall trend towards reduction in fat mass in non-responders, which potentially forms part of the HF progression. CRT success appears to be linked to maintenance of fat mass status at implant. The neurohormonal appears integral to this system. This is the principle hypothesis that should be investigated further.

7.7 PUBLICATIONS

*This chapter has formed the basis of a conference poster presentation at the Heart Rhythm UK conference in October 2016 (**Appendix R**). A review publication was published in the British Journal of Hospital Medicine in June 2016 sumarising the literature presented in the discussion and **chapter 1.7** (**Appendix S**).*

Chapter Eight

DISCUSSION

8.1 DISCUSSION

HF remains one of the greatest challenges to cardiovascular medicine for both the patient and wider society. The prevalence continues to increase and continues to be associated with poor outcomes.[2,313] Several pharmacological agents, specifically angiotensin receptor blockers[314], beta-blockers[13], mineralocorticoid receptor antagonists[11] and angiotensin receptor neprilysin inhibitors[315] have been shown to significantly improve morbidity and mortality. However, despite advances in medications the incidence and prevalence of HF continues to rise and confers a poor prognosis.[2,313] CRT has revolutionised management of HFrEF patients with cardiac dyssynchrony by improving mortality, reducing hospitalisation rates and inducing cardiac reverse remodelling.[22,23,26-28,31] However, a significant minority of patient 20-40% of patients who meet the implantation criteria fail to respond to CRT implantation.

Non-response is one of the greatest challenges of CRT. Predicting a response prior to CRT has remained a challenge despite extensive research in this field. The most important predictor of success has been shown to be QRS duration >132msec with a magnitude of increase in benefit for increasing duration, which is based on a large meta-analysis of five Medtronic RCTs.[39] Ruschitzka *et al*[41] in a RCT of CRT devices in patients with a QRS duration <130msec demonstrated a higher mortality in those with CRT devices on, to the point that the trial was stopped early. These observations have informed the new European Society of Cardiology 2016 implantation criteria to increase the lower boundary of QRS duration resting ECG for when to consider CRT implantation.[316] The evolving evidence is changing as to which patients are perceived to benefit from CRT. Bundle branch morphology of the QRS

complex on the resting 12 lead ECG has also been observed (in a recent meta-analyses) to predict response following CRT implantation.[44,45] Separating out the individual prognostic valve of bundle branch morphology from QRS duration is difficult as they appear to closely related to each other; LBBB is observed to be associated with a broader QRS durations.[39] Multiple other variables have been observed to predict response in different observational or post-hoc RCT studies (**Table 1.3**). However these observations are often not replicated. Predicitng non-responders remains an important goal for both the patient and society, given the cost and risk involved in implanting a CRT device.

Research into predicting response has been beset with challenges. Firstly there is no accepted definition, with many different definitions being used.[1] Fornwalt *et al*[1] observed that there was poor correlation amongst most definitions and no correlation between echocardiography and clinical definitions. Secondly, the majority of research in this field is in small observational studies, often not powered for the observations being made. The systematic review undertaken in **Chapter 4** highlights this particular limitation. Thirdly, the heterogeneity of the different HFrEF study population's means direct comparison between different analyses is often difficult. The limitations of the research in this field make study observations challenging to examine and validate. Success thus far in testing variables has only come when testing RCT datasets in a meta-analysis setting, but these still make response difficult to assess.

HFrEF is a heterogeneous condition, which develops as a result of myocardial injury from a several different potential (ischaemia, volume and pressure overloads etc) mechanisms which potentially result adverse cardiac remodelling.[113] Adverse cardiac remodelling leads to neurohormonal activation, pro-inflammatory changes, ECM remodelling and myocardial apoptosis.[94] Circulating biomarkers are abundant and reflect the changes that are undertaken in these systems during HF.[94] Alteration in circulating biomarker levels reflect the development and progression of HF and many have demonstrated the ability to predict cardiovascular outcomes.

The cardiac ECM is a highly dynamic support structure and actively remodels during the development and progression of HF.[94,113] Turnover of ECM alters with a pathological insult that leads to adverse remodelling of the myocardium. There is increased turnover with more degradation of exisiting collagen and deposition of newly constructed collagen, forming new ECM. The mechanism of injury to the myocardium determines the precise dynamic response of the ECM.[113] Spinale *et al*[113] summarises how specifically the ECM turnover increases in verying injury mechanisms. Broadly this varies between an ischaemic, pressure or voume overload injury mechanism. Degradation increases, allowing for the formation and deposition of new collagen. This process significantly contributes to the development and progression in HF. The MMP's are key regulatiors of the degradation of collagen, with many being specific for certain types. In HF ,MMP levels and activation have been shown to increase.[94,113,135] Progressively increased turnover of collagen leads to the development of cardiac fibrosis [113] Cardiac fibrosis development is a signficiant contributor to the adverse remodelling of the myocardium during HF. Increased turnover of the ECM

with progression to cardiac fibrosis leads to stiff ventricles, decreased relaxation and complicnace. [94,113] In turn these lead to the development of diastolic dysufunction, which in turn relates to systolic dysfunction.[113] The development of cardiac fibrosis respresents a poor prognostic feature for HF.[94,113] Specific circulating biomarkers reflect the cardiac ECM turnover of collagen, reflecting altered deposition (e.g. PINP and PIIINP) and degradation (e.g. CTx).[113] Increased PINP and PIIINP represent increased degradation of type I and III collagen respectively. More CTx in the circulation represents increased deposition of newly formed type I collagen. Cardiac ECM turnover is performed by specific enzymes (e.g. MMPs.-1,-2 and -9) and these are regulated by TIMPs (e.g. TIMP-1). Alterations of these specific circulating biomarkers have been shown to associated with adverse cardiac remodelling of HF and have been shown tp habe the ability to predict HF outcomes.[124,134,136,317] We hypothesised that certain levels of circulating cardiac ECM biomarkers reflect changes in collagen turnover in HF patients, therefore potentially have the ability to predict a patient's ability to respond to CRT implantation.

In **Chapter 4**, a systematic review was undertaken to examine all the cardiac ECM biomarkers that had been studied to test their ability to predict CRT response. A total of 6 studies, which researched a total of 9 biomarkers, met the criteria to be included in the systematic review. These included 5 observational studies[318] and 1 sub-study[119,125,244,294,295] of the CARE-HF[23] RCT. A meta-analysis was originally planned; however all the studies demonstrated large heterogeneity. There were 3 functional and 3 echocardiography response definitions in use within the included studies. The study designs and patient population's also contrasted with each other making amalgamation of the studies difficult.

All the studies were small in terms of participant numbers and none were powered. The studies reported differences in the behaviour of these biomarkers and differences in expression predicting response (**table 4.6**). The literature to date demonstrates that no consistent pattern of behaviour of the ECM has been demonstrated to predict response. The definition used is different between each study; therefore the circulating biomarker expression level is being compared to different response definitions. However, even in the studies where changes in ventricle volumes and function are made the definition, there are different reported patterns of ECM circulating biomarker expression. This means no clear pattern of behabviour on HF patients has been determined to date. These studies with common respond definitions are often not comparable because os study design and cohort characteristics differences. To exert real consistent valve in these observational studies a common definition should be applied to a specific population. Logistically, however this would pose a real challenge as it would be more difficult to recuit participants and would require more centres being involved.

MiRNA's are short endogenous non-coding RNA which regulate protein expression at the post transcriptional level.[139] MiRNA's are recognised as regulators of complex biological systems and many are tissue specific.[139] They are stable in the circulation and considered to have great potential as biomarkers.[143] Multiple miRNA have been observed to be dysregulated in HF and have potential as biomarkers.[158,188,191] **Chapter 1** summarises the miRNA that have been studied in HF. Variability in study designs, laboratory techniques, HF patient's characteristics and small participant numbers have meant that observations are often not replicated consistently.[139] Marfella *et al*[197] and Melman *et al*[199] have observed

miRNA's potentials as biomarkers in predicting response in patients undergoing CRT. There remains limited evidence to validate their use in clinic prwctive. The current evidence is limited by studies only on small cohorts, variation in quanitification technique and hetrogenity of study populations.

Predicting non-response in patients undergoing CRT implantation is important to both the patient and society. Building a prediction model that improves our prediction rate is important to these HF patients to allow them to make better informed decisions. What the research to date has shown us is many variables are important on an individual basis, but predicting a populations chances of a response is difficult due to the heterogeneity of the condition. Building a model involves examining several important variables and examining those that have potential. We hypothesised alongside certain clinical variables that ECM biomarkers and specific miRNAs that have shown dysregulation in HF may be important predictors that have not been fully assessed. This body of research aimed to undertake a small observational study to test this hypothesis as a proof of concept study.

In, **Chapter 5** all the implant data from out single centre was examined (2009-2013) to explore our potential cohort and examine variables that may be important to account for in our prediction model when examining the selected circulating biomarkers. Certain variables based on the literature outlined in **Chapter 1** would be included in the prediction model to account for factors that are known to predict response. Examining the characteristics of our heterogeneous group of patients we have implanted at UHCW reflects a unique population

(T**able 5.2**), which will vary from other centres. These characteristics were important to understand when planning study II (COVERT-HF) to examine circulating biomarkers. The retrospective cohort study also allowed us to examine clinical (symptoms only) response and to see if any pre-defined variables (**Chapter 3**) were important within our cohort. The only variable that was shown to predict a clinical response was age at implant, reflecting a 4% chance for every year older at time of implant (**Table 5.5**). These findings are driven by those patients who had their clinical response assessed over 12 weeks following implant. Importantly QRS duration and bundle branch morphology were not shown to be predictors of response, which is somewhat surprising given the previous reported strength to predict response/outcomes. These results are due to the small numbers of patients and the response definition being assessed over a wide period of time. There was also a proportion of missing data, accounted for some patients being referred from other centres in the Arden Cardiac Network and availability of information. Pre-defined clinical variables were also analysed to test their ability to predict cardiovascular outcomes (MACE and/or first HF hospital admissions). The presence of CKD at baseline (estimated GFR <60 ml/min/1.73m^2) predicted the composite cardiovascular outcome following CRT implantation (HR 2.10, CI (95%) 1.23-3.19, *p*=0.001) in a multivariate regression model (**Table 5.7**). The CKD findings replicated previously reported poor outcomes for CKD patients following CRT implantations.[238,300] Both age and CKD status were important in our cohort and would be accounted for in the prospective study prediction model.

In **Chapter 6,** we report the ability of ECM (PINP, CTx, PIIINP, MMP-2 and MMP-9) and miR (-21,-30d,-122,-133a,-210 and -486) circulating biomarkers to predict a single HFrEF patients

ability to have a functional response following CRT implantation (**Figure 6.4**). Alongside these biomarkers six important variables (QRS duration,[39] BBB morphology,[45] CKD,[238] gender[306] and aetiology[306]) were analysed for their predictive ability. GDF-15[112] and NT-*pro*-BNP[100] biomarkers were also included in the prediction models given their previous reported abilities to predict response. These results that none of the biomarkers actually predict functional response are supported by the observations reported by Lopez-Andres *et al.*[125]

Our proof-of-concept prospective observational study recruited a small heterogeneous cohort (82.7% male, 72.4 mean age, 57.7% ischaemic aetiology, 51.9% NYHA III, 36.5% AF and 75.0% LBBB) over two years (Nov 2013- June 2015). The multivariate logistic regression model did not observe that any variables could predict functional response in participants undergoing CRT (**Figure 6.4**). The result indicated that increasing baseline CTx expression and LBBB morphology trended towards predicting functional response. The CTx results indicate that increased turnover at baseline may reflect the increased ability to respond to CRT if there is more dynamic turnover of the ECM. Mechanistically, this suggests those patient's further along in the development of cardiac fibrosis have the greatest potential to improve when a CRT is implanted. Therefore, potentially if these changes are seen across the entire left ventricle, the diastolic function might improve compliance and decrease stiffness allowing for an improvement in symptoms (NYHA) and exercise tolerance (6MWT distance). CRT implantation may be able to exert a greater influence on those patients whom are still actively remodelling at the molecular level with increased ECM turnover. Therefore, patients with increased formation and deposition of collagen may have the

greatest potential chance to benefit from CRT implantation. The correlations between changes in PINP and PIIINP with 6MWT distance at 6 weeks favours this conclusion. PINP has also been observed to have increased expression in functional responders following CRT implantation (**Figure 6.2**). Highlighting the influence CRT implants has on ECM turnover. However, contrary to this observation no difference is seen between responders and non-responders for the other tested circulating biomarkers.

Our results however do not completely support this mechanistic conclusion. The PINP and PIIINP baseline results showed no difference between responders and non-responders. There would be an expectation that CTx levels would vary between those that reverse remodelled (↓ ≥15% LVEDV at 6 months) on echocardiography following CRT implantation as reversal of ECM turnover would lead toventricular reverse remodelling. However, no difference between echocardiographic responders and non-responders were observed for any of the studies circulating biomarkers.

The study (**Chapter 6**) did not indicate any variation in the expression of ECM or miRNA biomarkers patients with and without MACE events (**Table 6.5**). No definitive observation can be made that ECM expression or miRNA dysregulation predicts functional response based upon the COVERT-HF study. Higher expression was noted of ECM biomarkers systemically than in the coronary sinus, whereas the specific miRNA selected for study were found to have higher expression in the coronary sinus (**Figure 6.5**). The regulation of cardiac ECM turnover has systemic involvement, but the dysregulations of miRNA appears to occur

in the heart. There are however suggestions that alterations in ECM turnover on type I collagen may be an important observation between responders and non-responders, whether this may be useful in future clinical studies requires further and more refined studies.

In **Chapter 7**, consideration was given to body composition and CRT in HFrEF patients in a small physiological pilot study (COVERT-HF sub-study). There is a complex interplay between body composition, metabolic processes and HF (**Chapter 1**).[218] These have been extensively studied and describe the influence of body composition on metabolic dysregulation that occurs during HF development and progression.[218] Advancing HF favours the development of a pro-catabolic metabolic state and this is demonstrated by the changes in the neurohormonal, inflammatory and ECM pathways previously discussed. The progression towards a catabolic state favours poorer cardiovascular outcomes.[218] Body composition has been shown to exert influence on the development and progression of HF through these metabolic pathways. The presence of adiposity in established HF exerts a protective effect on patients.[206,218] The understanding of this complex interplay is not fully understood. There is limited research about body composition behaviour and its influence on HFrEF patients following CRT implantations. Only a Chinese observational study to date has examined the impact differing BMI at implantation has on response and cardiovascular outcomes, suggesting the presence of adiposity at the time of CRT implantation favours a better response/outcome.[216]

Chapter 7 reported the observtaions of the effects the implant of a CRT in a HFrEF patient had on body composition and and whether there was any difference between functional responders and non-responders. No differences were observed between functional responders and non-responders at implant (**Table 7.1**). Following CRT implantation, body composition did not significantly vary for the entire cohort over 6 months (**Table 7.2**). Furthermore no significant difference was observed between responders and non-responders (**Table 7.3** and **Figure 7.2**). However, there was a trend towards a reduction in fat mass immediately following CRT implantation. There was also a strong correlation between changes in fat mass and LV geometry, suggesting that a reduction in fat mass was related to an increase in LV dilation over the first 6 weeks following CRT implantation (**Figure 7.3**). This observation supports those previously reported in the literature about the protective effect of adiposity on HF patients. Due to the size of the study, these observations can only be hypothesis generating, but certainly pose an area of further research. However, they may also overstate the relationship due to the limited available data. The correlations curves are likely to more heavily impacted by outliers, due to the low number of data points.

8.2 LIMITATIONS

This body of work has several key limitations that must me acknowledged when drawing conclusions on our observations and utilising them to inform the next steps of research into predicting response following CRT implantation. Initially the challenges of research in this field should be discussed as our research did not escape them. The greatest challenge is the inconsistency in definitions applied throughout research in this field for a response to CRT.

Fornwalt *et al*[1] has identified when comparing the commonest definitions there is often very poor correlation. Our own critical evaluation in the systematic review (**Chapter 4**) of the research into ECM biomarker potential as predictors of CRT response demonstrated the wide variation in definitions used (**Table 4.2**). This research informed the decision to use a composite functional definition rather than also utilising an echocardiographic criterion for the prospective study. Making the decision for the best definition for the prospective study meant our retrospective study (**Chapter 5**) was not directly comparable to the prospective observational study (**Chapter 6**). In the wider literature our primary results are only directly comparable to those with functional definitions, which ultimately further highlight the challenge of not having a universal response definition.

The systematic review (**Chapter 4**) was a critical review of the literature that informed the decision behind the selection of the cardiac ECM biomarkers. The initial aim was to undertake a meta-analysis and pool the analyses. Unfortunately, this was not possible given the heterogeneity of the research studies included. Firstly, the studies represented a wide HFrEF population that varied between the studies. Secondly the study designs differed significantly between each other, including the period of time the participants were observed for. Thirdly, the definitions of response differed so significantly (**Table 4.2**) that response assessments were not comparable. Finally the studies included were all small and not powered. Critical appraisal was undertaken however a complete amalgamation of the results and a meaningful meta-analysis was not possible.

The retrospective registry (**Chapter 5**) had several critical limitations which are important to address. The number of anonymous patients involved was limited, though comparable to similar studies.[64,65] There was a small degree of missing data due to incomplete records or patients having limited information available as they were referrals from outside UHCW. Information was requested and collected from these sites, but despite strenuous efforts it was often incomplete. Multiple imputation techniques were used to overcome these limitations, but they may account for QRS duration and BBB morphology not being shown to be predictors when this is clearly the case if the literature. The study was also under-powered for the findings that were identified. There was also a possible reporting bias, due to the fact the two clinicians assessing clinical response were colleagues, despite stringent efforts being made to avoid collusion.

The COVERT-HF study had several limitations that need to be explored. Firstly, during participant recruitment the NICE 2007[51] implantation guidelines were altered in 2014. An echocardiographic metric of dyssynchrony was removed from the 2007[51] guidelines. Recruitment occurred under both the 2007[51] and 2014[17] guidelines. The inclusion criteria for COVERT-HF reflected the NICE implantation criterion. We aimed to produce a cohort as close to 'real world' as possible, so altered the inclusion criteria to reflect the 2014 guidelines. However, this alteration may reflect a change in the cohort recruited before and after this point. Secondly, there have also been developments in the pharmacological therapy for HF recently with the introduction of angiotensin receptor neprilysin inhibitors (ARNI) following the 'Prospective comparison of ARNI and ACEi to Determine Impact on Global Mortality and morbidity if Heart Failure trial' (PARADIGM-HF), which showed a 16%

relative risk reduction in all-cause mortality.[315] This new therapy may impact outcomes and response rates for CRT therapy, which means our results, may not be as current as they do not include any patients on ARNIs. Thirdly, the study was single centred, with small participant numbers and it was not powered, which means the observations made should be questioned and may represent statistical chance. This may also mean that just because an observation is not made in the COVERT-HF study it does not mean it does not exist. Fourthly, not all patients were able to have a complete echocardiogram performed, due to either missing a visit or having poor quality images. This may produce a reporting bias for the echocardiographic results. Fifthly, ECM biomarkers altered expression is attributed to HF, however the cohort was older and had other health issues, which may affect the systemic expression of these biomarkers. Finally, the CS samples could only be taken in the latter half of the cohort. There were a reduced number of paired results due to a high haemolysis rate of the CS samples. The CS sample results must be scrutinised due the small number of results made.

The body composition analysis examined the hypothesis that body composition is effected by CRT implantation in HFrEF patients. It then explored whether there was a difference between responders and non-responders to the degree it might be a potential predictor. Therefore, the value of this sub-study to the prediction model is limited, but it adds value for the future work to be considered. The sub-study occurred in a small sub-set of the cohort study. The participant numbers were not large enough to stratify patients by a body composition metric, an option that should be considered for the next area of study. Air-displacement plethysmography is a non-invasive option of measuring body composition that

was acceptable with an implanted cardiac device. The limitation of this body composition measurement method is it is not validated in HF patients and the presence of body water is interpreted as fat mass. This means that patients with fluid retention are measured as having a higher fat mass. A further limitation of this sub-study was the reporting bias of patients having incomplete echocardiograms performed. The most frequent reason for sub-optimal images was a large body habitus, meaning that participants with larger fat masses were more likely to have absent useable echocardiography data. This is likely to represent a reporting bias.

8.3 FUTURE WORK

Our research demonstrates that predicting response involves multiple factors and their interactions. Predicting response is likely to take a more individual approach focusing on a variety of potential risk predictors. Our body of research reviews the evidence behind well-established predictors like QRS duration and BBB morphology. However, these observations have not changed the significant non-response rate. Tailoring the prediction model is the direction the research in this field has been moving.

Our research replicated in our retrospective registry that CKD status is an important variable in predicting outcomes following CRT implantation. Together with the review of the previous literature, important potential predictors were accounted for in a prediction model that focused in the COVERT-HF study on pre-selected ECM and miRNA biomarkers as important variables. Informed and critical reviews identified biomarkers that had potential

to predict a response. This potential model was applied to every participant undergoing CRT implantation. This meant the cohort was very heterogeneous and reflected the broad swathe of different patients suffering from HFrEF. Our results did not show a definitive predictive value to the ECM and miRNA biomarkers tested, but there was a suggestion Type I Collagen turnover maybe important. However, the COVERT-HF study did not demonstrate this.

For a future direction of research, a larger scale multi-centre should be considered to test whether baseline type I Collagen turnover biomarkers (PINP and CTx) are predictors of response to CRT. To demonstrate a significant difference between baseline concentrations in PINP and CTx as predictors of response (non-response rate 42.3%) a sample size of N=430 HRrEF patients was required. Assuming an 80% power and a significance level of p=0.05. However, a more focused strategy should also be considered for predicting response. Minimising the heterogeneity of the study population is more likely to reveal important predictors. For example focusing on a patients HF aetiology and studying only ischaemic or non-ischaemic patients might yield a more consistent pattern of ECM biomarker behaviour. The ECM remodelling following a similar cardiac injury would be more likely to behave and respond more consistently following CRT implatation.[113] A study examining patients who meet CRT implantation criteria and who have the same aetiology for there HF would be required to minimise heterogeneity, however it would take more time to recruit the same number of participants. A multicentre approach would overcome this challenge, but require more funding.

The body composition sub-study has produced the physiological hypothesis that more adiposity favours improved reverse cardiac remodelling following CRT implantation. This hypothesis needs to be tested to see if body composition should be considered before implanting a CRT. Further observation studies should be undertaken, that are powered to observe differences in participants response and outcomes based on their baseline relative fat mass or lean mass. If proven to be important, these body composition metrics should then be tested as potential predictors of response. CC should also be observed to see the differences following CRT implantation on response/outcomes in those where is present or absent. Prior to implantation CC should be screened for and participants stratified based on its presence and absence. Furthermore, if it transpires to be important, CC should be tested as a potential predictor of response.

8.4 CONCLUSIONS

CRT is a game-changing development in the management of HF patient's refractory to OMT that improves survival and decreases morbidity. Unfortunately, non-response occurs in a significant minority, impacting individuals and wider society. Important work has taken place examining potential predictors of response, the most important of which is QRS duration and BBB morphology, which have now altered the implantation criterion.[316] However, non-response remains one of the greatest challenges in HF management. Specific ECM and miRNA biomarkers which have been observed to be important in HF have not demonstrated the ability to predict functional response. However, circulating biomarkers of type I collagen turnover are observed to have the potential to predict functional response. A larger cohort study could potentially test this hypothesis. The key achievement of this body

of research is to highlight the heterogeneity that challenges research into testing potential CRT predictors, especially ECM and miRNA biomarkers. Adapting research designs to compensate for heterogeneity will go some distance to being able to absolutely test a biomarkers ability to test potential to predict CRT response. Furthermore, body composition maybe important in prediction models and further study is required. In combination with other predictors involved in systematic remodelling it may be an important predictor of CRT response and outcomes.

Chapter Nine

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APPENDIX A

Systematic Review Protocol

Study Protocol

Cardiac Extracellular Matrix Markers in Reduced Ejection Fraction Heart Failure Patients treated with Cardiac Resynchronisation Therapy as Predictors of Response and Outcome: A Systematic Review

Version 2: 13th February 2016

1.0 Introduction

CHF is a common, costly and disabling condition affecting almost 1 million people in the UK.[2] CRT is one of the most effective heart failure therapies to emerge in the last 25 years and is applicable to a third of all symptomatic heart failure patients.[234] It involves implantation of pacemaker leads to pace the right atrium, right ventricle and left ventricle (via the CS) to resynchronise cardiac contraction. Several prospective randomised studies have shown that CRT is associated with a significant reduction in hospitalization rates for heart failure and improved long-term survival.[22,23,28] Consequently, CRT has gained widespread acceptance as a safe and effective therapeutic strategy.

Recently indications for suitable patients have been broadened based on more focused evidence of patients that may benefit from CRT implantation.[17,38,319] The new guidance requires a LVEF≤35%, optimal medical therapy, in either sinus rhythm or atrial fibrillation (where satisfactory level of biventricular pacing can be achieved). All NYHA symptoms classification can be considered; I (QRS>150msec on resting ECG), II/III/IV (QRS>150msec or 120-149msec with LBBB)[17,47,56]. Patients undergoing pacemaker implantation or upgrade should be considered for CRT if LVEF≤35% and likely to be pacing dependent >40% of the time[47]. However, despite these indications, a significant proportion of patients (approximately 20-30%) remain unresponsive and have recurrent hospitalisations for heart failure with no improvement or even deterioration in symptoms with CRT pacing.[23,27,28,31,41,250,319] As such, better identification of suitable patients would be of great benefit to the NHS.

Multiple biomarkers have been associated with different disease processes within CHF and have been examined for their clinical value. Van Kimmende *et al*, outlined the multiple circulating biomarkers that have been associated with CHF and alter with development and progression of the condition, highlighting their potential clinical value. [94] Table 1 demonstrates the circulating biomarkers highlighted by Van Kimmende *et al* as altering in CHF. Currently only N-terminal *pro*-brain natriuretic peptide (NT-*pro*-BNP), a neurohormone is utilised clinically in diagnosis and prognostication of CHF. [2,100,320]

ECM is of particular interest in heart failure development and progression, given the dynamic nature of its structure and function. [113] Implications for ECM in the major injury mechanisms of the myocardium in the development of all types of heart failure have been examined. [113] Critically the implication on the signalling of progression has been described and the potential development of myocardial fibrosis.[113] Changes in structure and function of ECM have been associated with clinical

outcomes.[94,113,115,132] Surrogate markers are available for the key structural collagens and the metabolic processes involved in regulating ECM in the blood stream and these have been highlighted as potential biomarkers of the development and progression of heart failure. [94] Table 1 demonstrated ECM biomarkers, that have previously been studied in HF.[94]

CRT therapy for patients outlined in national guidelines has been demonstrated to improve LV geometry and clinical outcomes for CHF patients.[17] Reverse remodelling of the LV occurs in many CHF patients following CRT, which is demonstrated by improved LVEF, decreasing LVEDV and functional mitral regurgitation.[22,23] LV reverse remodelling is associated with changes in ECM structure and function. [119,120,126,263,298] ECM remodelling offers a potential marker of prognosis following CRT and much work has been done to determine the potential clinical value of circulating biomarkers of ECM on predicting CRT response and/or MACE.[119,120,126,244,321] These studies have tended to be small observational studies or post-hoc RCT trial analyses and do not collectively demonstrate a clear pattern. ECM and its surrogate markers have the potential to demonstrate the LV's future ability to reverse remodel and future clinical response to CRT implantation.

Table 1: Emerging Biomarkers in Heart Failure. ***Van Kimmende et al (2012)***[94]

Inflammation	Myocyte stress	Extracellular-matrix remodelling	Neurohormones
CRP Norepinephrine	BNP, NT-proBNP, MR-proANP	MMPs	Norepinephrine
TNF-	sST2	TIMP1	Renin
TWEAK (TNF-like weak inducer of apoptosis)	Growth Differentiation Factor 15	IL-6	Angiotensin II
IL-1, 6, 10, and 118	Extracardiac involvement	Collagen propeptides	Aldosterone
LP-PLA2	RDW	N-terminal collagen type I/III peptide	Arginine vasopressin, copeptin
Soluble TNF receptors 1 and 2	Cystatin-C, -trace protein	Myostatin	Endothelin-1
YKL-40 Urocortin	NGAL, NAG [N-acetyl--(D)-glucosaminidase]	Syndecan-4	Urocortin
IL-1 receptor antagonist	KIM-1 (kidney injury molecule-1)	Galectin-3	Chromogranin A and B
Midkine	ß2-microglobulin		MR-proADM
Leucine-rich 2-glycoprotein	Urinary albumin-to-creatinine ratio		
PTX3	Triiodothyronine		
CA-125		**Myocyte injury and apoptosis**	**Oxidative stress**
S100A8/A9 complex		Troponins I and T	Oxidized LDLs
Osteoprotegerin Creatine kinase MB fraction		Myosin light-chain kinase I	MPO
Serine protease PR3		Heart-type fatty-acid–binding protein	Urinary biopyrrins
Soluble endoglin		Creatine kinase MB fraction	Urinary and plasma isoprostanes
Adiponectin		sFAS (soluble apoptosis-stimulating fragment)	Urinary 8-hydroxy-2-deoxyguanosine
		Heat shock protein 60	Plasma malondialdehyde

Adapted from van Kimmenade *et al* [94]

2.0. Objective Systematic Review

The objective of this systematic review is to consolidate all studies relating to ECM surrogate circulating biomarkers (table 1) on their ability to predict response (clinical/echocardiographic) and MACE in CHF patients undergoing CRT implantation with the following. The primary objective of this systematic review is:

1. What is the value of the ECM biomarkers van Kimmenade *et al* [94] listed at predicting patient response (clinical/echocardiographic) to CRT?

Secondary objectives for this systematic review are:

1. What is the value of the ECM biomarkers van Kimmenade *et al* [94] listed at predicting patient MACE following CRT implantation?
2. To report the definitions of 'response' used within the ECM biomarker and CRT literature.
3. Does the pattern of ECM biomarker expression following influence response and MACE prediction?

3.0 METHODOLOGY

This systematic review protocol has been produced according to the Preferred Reporting Items for Systematic Reviews and Meta-analysis Protocol [PRISMA-P 2015][288]. The review is registered with the systematic review website PROSPERO [25864]. The systematic review will be conducted in-line with the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines.[287]

3.1. Eligibility Criteria

Study selection for inclusion in the systematic review must meet these strict eligibility criteria.

3.1.1. Target Population

CHF patients that meet specific CRT implantation criteria outlined in the international guidance.[17] [47,234]

3.1.2. Circulating biomarker

All ECM circulating biomarkers outlined by van Kimmenade e*t al* [94] (table 1) will be included in the systematic review as an association with CHF has previously been described.[94] Biomarkers must be

taken peripherally when the patient is clinically stable, up to seven days prior to implantation. Principle focus of the articles included in the review will on pre-implant ECM biomarkers. Post CRT implantation ECM biomarker analysis will only be included if the pre-implant are examined for their predictive value.

3.1.3. Outcomes

Multiple clinical/echocardiographic response and MACE definitions have been used in literature as a metric of CRT success.[1] Correlations between different definitions is often poor.[1,100] Comparison between different studies of a particular biomarker or intervention is often difficult due to the different response/MACE definitions used. It is recognised that this is a limiting factor of performing a systematic review within this field. Application of a clinical response definition will be undertaken as the primary outcome measure of the systematic review for ECM biomarkers listed in table 1. To be included in the analysis, the articles must measure clinical and/or functional and/or quality-of-life data at baseline and final follow-up (>6months post implant). Separately the degree of echocardiographic change in LV geometry between implant and follow-up (>6months post implant) will form a separate response definition to be evaluate in the review. MACE will be defined as all-cause mortality and/or first heart failure hospital admission during observation period. Absence of secondary outcome data will not exclude an article from being included in the systematic review.

3.1.4. Selection of Articles

All types of cohort and randomised control trials (including post-hoc analysis) will be included in the systematic review. The study must be on adults only (ages ≥18) and performed principally to examine clinical value/outcome measures. Translational research will not be included. Review articles will be excluded.

3.2. Database Search Strategies

Detailed searches will be conducted on PubMed, Ovid SP MEDLINE, Cochrane Library (CENTRAL) and TRIP. The search strategy will be designed and undertaken by one author [CM] and reviewed by another independently [DA]. The bespoke search strategy will search for the specific terms '*cardiac resynchronization therapy*'/'*cardiac pacing*'/'*extracellular matrix*' in combination, within titles/ abstracts or Medical Subject Headings (MeSH). Moreover the specific circulating biomarkers (*'TIMP' 'MMP' 'collagen' 'Myostatin' 'Syndecan-4' and 'Galectin-3')* will be included in the search strategy. Specific TIMP, MMP and Collagen circulating biomarkers will not be searched for as these will be identified using the broader classification terms. Clinical Trials (www.clinicaltrials.gov) will be

searched for ongoing or unpublished work relating to the systematic review. The largest international cardiology conferences (European Society of Cardiology, American Heart Association and the American College of Cardiology) will undergo a manual search of abstracts. A paper sift search will be performed on all papers taken forward to full review. A date limitation of the last 15 years (31/12/1999 – 31/12/2015) will be applied to the search strategy to coincide with the advent of CRT as a mainstream therapy. No language restrictions will be applied to the search strategy.

Title/Abstract reviews will be performed independently [CM and DA] and those that meet eligibility criteria will be taken forward for full paper review. Consensus must be reached to take abstract forward to full paper review, any conflicts will be adjudicated by a third independent reviewer [FO]. Duplications of articles will be identified and only one will be taken forward to full paper review. It is anticipated that several conference abstracts will be identified as articles separately. Articles in this instance will only be taken forward. Each article will undergo full paper review to ensure compatibility with the eligibility criteria. Full article reviews will be undertaken using the Criticial Appraisal Skills Programme checklist dependent on study design to ensure quality of assessment to match the eligibility criteria for the systematic review.[289] Consensus must be reached on full paper review to take study forward into data extraction, any conflicts will be adjudicated by a third independent reviewer [FO].

3.3. Data Extraction and Management

Full texts of the articles included in the analysis will be retrieved. A standardised data extraction will be piloted on two separate articles and then reviewed for its robustness [CM, DA, FO, PB]. The standardised data extraction form will collect data on study design (number of participants, eligibility criteria, study design, assessment period), patient population (age, gender, aetiology, ECG, LV geometry, quality of life, NYHA, functional assessment), circulating biomarker / predictor (specific ECM surrogate circulating biomarkers; units measure, conditions taking samples, laboratory assessment, statistical assessment) and outcome (response definition and MACE. Data extraction will be performed by two independent reviewers [CM and DA]. A third independent reviewer [FO] will resolve any disagreement. All authors of papers taken forward to each review will be contacted for further details and available data. In situations where more than one article is published off the back of one dataset, no direct comparison will be performed between the articles, but will be included if different ECM biomarkers are examined in each article.

3.4. Risk of Bias Assessment

The risk of bias for each study will be assessed by two reviewers [CM and DA] independently utilising either the Cochrane Collaboration 'Risk of Bias' assessment tool or the Risk of Bias Assessment Tool for Nonrandomised Studied [RoBANS], whichever is the most appropriate per the particular study [290,291]. Both these standardised risk assessment tools have established criteria to examine selection bias, exposure measurement, blinding and completeness of outcome data.

3.5. Data Synthesis and Analysis

Individual trials data will be presented summarising findings for each ECM biomarker. Datasets will be sought from the corresponding authors. Distribution of outcome measures will be summarised. Overall cohorts for each ECM biomarker will be compared too. When a different studies use the same outcome definitions at similar time-points a comparative analysis will be performed. The broad definition of outcomes is recognised as being a limitation of the study.

4.0 Discussion

CHF as a condition carries a high mortality as it progresses following diagnosis. Changes in LV geometry are a hallmark of progression and poor outcomes in CHF. Medical treatments for heart failure have significantly improved outcomes. [2] CRT is an effective therapy for treating CHF and improves outcomes and reverse remodels the LV. [22,23,38] Despite improvements in CHF with CRT, 30% continue not to respond to treatment. [235,322] The focus of research at the moment is examining ways to better predict response clinically, with several options demonstrating promise, but not quite changing practice.

ECM surrogate circulating biomarkers represent changes in a dynamic structure that remodels with continued myocardial injury, altering cardiac structure and function.[113] In particular this causes LV geometry alterations, associated with poor outcomes. ECM surrogate markers have been associated with diagnosis and progression of CHF. [94]

Several biomarkers have been examined to determine clinical value in CHF patients undergoing CRT implantation, but none have demonstrated a clear benefit clinically. ECM surrogate markers have been particularly scrutinised and offer a potential tool to predict outcome following CRT treatment. Unfortunately the narrative is not clear with evidence not quite supporting each other. [119,120,126] One particular observed issue is that the studies tend to be small observational studies using different outcome definitions to determine response. Other studies are post-hoc analysis of big randomised

control trials. Study design is subtly different, making direct assessment difficult. Thus analysing ECM surrogate makers clinical is truly challenging.

This protocol outlines the proposed mechanism to bring all the evidence together to determine the clinical utility of these markers on ECM circulating biomarkers. The application of the protocol will be robust and clear.

5.0 Acknowledgements

The authors would like to acknowledge Petra Meeson (UHCW Library services) for her support and advice in setting up the systematic review.

APPENDIX B

Literature Search for Systematic Review

PubMed 15th February 2016

The search algorithm is given below, alongside the first two abstracts identified. The rest of the abstracts found in this search are not given in this appendix. If they are needed to be reviewed, they are available on the PROPSERO website.

73 results generate

Sent on: Mon Feb 15 07:22:57 2016

Search: ((((((tissue inhibitor of metalloproteinase) OR Matrix metalloproteinase) OR collagen) OR Myostatin) OR Syndecan-4) OR Galectin-3) OR ((extracellular matrix[MeSH Terms]) OR extracellular matrix[Title/Abstract]) AND ((((((((cardiac resynchronization therapy[MeSH Terms]) OR cardiac resynchronization therapy[Title/Abstract])) OR ((cardiac pacing, artificial[MeSH Terms]) OR cardiac pacing[Title/Abstract])))))

Search restricted 31/12/1999 – 31/12/2016

PubMed Results

Items 1 - 91 of 91 (Display the 91 citations in PubMed)

1. Heart Rhythm. 2016 Jan 8. pii: S1547-5271(15)01671-9. doi: 10.1016/j.hrthm.2015.12.036. [Epub ahead of print]

   Prognostic value of collagen turnover biomarkers in cardiac resynchronization therapy: A subanalysis of the TRUST CRT randomized trial population.

   Sokal A[1], Lenarczyk R[2], Kowalski O[2], Mitrega K[3], Pluta S[2], Stabryla-Deska J[2], Streb W[2], Urbanik Z[2], Krzeminski TF[4], Kalarus Z[2].

   CONCLUSION:

   Low PIIINP levels are associated with favorable echocardiographic response and long-term survival in CRT recipients.

   .

APPENDIX C

Applied Medical Definitions

Ischaemic Aetiology

Aetiology of reduced ejection fraction heart failure will be defined as ischaemic or non-ischaemic cardiomyopathy. Ischaemic cardiomyopathy will be defined as either: previous myocardial infarction, previous coronary bypass grafting, significant ischemic disease with previously treated stenosis of ≥50% of lumen diameter in ≥1 major epicardial coronary artery or cardiac magnetic resonance imaging defining ischaemic aetiology. Non-ischaemic aetiology are all those HFrEF patients that do not meet the above criteria.

Cardiomyopathy

Cardiomyopathies are defined by structural and functional abnormalities of the ventricular myocardium that are unexplained by flow-limiting coronary artery disease or abnormal loading conditions.[323]

Diabetes Mellitus

Diabetes will be defined as either patients on therapy [anti-diabetic therapy or insulin], a random venous plasma glucose concentration ≥11.1 mmol/l, a fasting plasma glucose concentration > 7.0 mmol/l (whole blood ≥6.1mmol/l), a two hour plasma glucose concentration ≥11.1 mmol/l two hours after 75g anhydrous glucose in an oral glucose tolerance test (OGTT) or HbA1c ≥48 mmol/mol (6.5%) [324].

Chronic Kidney Disease

Chronic kidney disease was defined as a GFR of ≤60 mL/min/1.73 m2 (estimated by modification of diet in renal disease equation).[237,239] **Table C1** outlines Stages of Chronic Kidney Disease based upon estimated eGFR.[239]

Table C1 Chronic Kidney Disease Stage. (Adapted[239])

Stage	GFR*(ml/min.1.73m^2)	Description
1	≥90	Normal kidney function
2	60-89	Mildly reduced kidney function
3	30-59	Moderately reduced kidney function
4	15-29	Severely reduced kidney function
5	≤15 or on dialysis	Very severe or end-stage kidney failure

- All GFR values are normalised to an average surface area of 1.73m^2

APPENDIX D

Retrospective Cohort Study I Data Collected.

Pre-Procedure

Demographics

1. Age at implant
2. Gender

Medical History

1. Aetiology Cardiomyopathy
2. Previous Myocardial Infarction
3. Previous PCI
4. Presence previous Coronary Artery Bypass Grafting
5. Angina
6. Diabetes Mellitus
7. Chronic Kidney Disease
8. Atrial Fibrillation

Medication (Pre-Procedure)

1. Heart Failure Medication

Pre-Procedure Electrocardiogram

1. QRS duration (msec)
2. BBB

Pre-Procedure Transthoracic Echocardiogram

1. LVEF (Modified Simpson's Biplane Method)

Procedure

1. Elective or Urgent Procedure
2. Type of CRT device
3. De novo or Upgrade device
    a. If upgrade what the index device was?
4. LV Lead circumferential position
5. LV Lead axial position
6. RV lead position (anatomical)
7. RA lead position (anatomical)
8. Failed Procedure (and reason)
9. Complications

Latest Review /Outcomes

1. MACE
2. Hospitalisations
3. All-Cause Mortality

APPENDIX E

Ethical Approval

Approval Letter

30 October 2013

Dr Faizel Osman
University Hospitals Coventry and Warwickshire (UHCW) NHS Trust
Clifford Bridge Road
Coventry
CV2 2DX

Dear Dr Osman,

Study title:	**The characterisation of circulating biomarkers before and after cardiac resynchronisation therapy in patients with heart failure and their role in predicting response**
REC reference:	**13/WM/0355**
IRAS project ID:	**135985**

Thank you for your letter of 28 October 2013, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

We plan to publish your research summary wording for the above study on the NRES website, together with your contact details, unless you expressly withhold permission to do so. Publication will be no earlier than three months from the date of this favourable opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to withhold permission to publish, please contact the REC Manager, Helen Wakefield, NRESCommittee.WestMidlands-Edgbaston@nhs.net.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Non-NHS sites

The Committee has not yet been notified of the outcome of any site-specific assessment (SSA) for the non-NHS research site(s) taking part in this study. The favourable opinion does not therefore apply to any non-NHS site at present. We will write to you again as soon as one Research Ethics Committee has notified the outcome of a SSA. In the meantime no study procedures should be initiated at non-NHS sites.

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database within 6 weeks of recruitment of the first participant (for medical device studies, within the timeline determined by the current registration and publication trees).

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non clinical trials this is not currently mandatory.

If a sponsor wishes to contest the need for registration they should contact Catherine Blewett (catherineblewett@nhs.net), the HRA does not, however, expect exceptions to be made. Guidance on where to register is provided within IRAS.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Advertisement		
Covering Letter		13 August 2013
GP/Consultant Information Sheets	2.0	02 September 2013
Investigator CV	Dr Faizel Osman	13 August 2013
Investigator CV	Dr Christopher McAloon	
Letter of invitation to participant	4	22 October 2013
Participant Consent Form	5	07 October 2013
Participant Information Sheet	5	07 October 2013
Protocol	4	14 July 2013
Questionnaire	2	03 September 2013
REC application	135985/488787/1/948	13 August 2013
Response to Request for Further Information		26 September 2013
Response to Request for Further Information		28 October 2013

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

<u>Reporting requirements</u>

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

Further information is available at National Research Ethics Service website > After Review

13/WM/0355 Please quote this number on all correspondence

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

With the Committee's best wishes for the success of this project.

Yours sincerely,

Mr Paul Hamilton

Chair

Email:NRESCommittee.WestMidlands-Edgbaston@nhs.net

Enclosures: "After ethical review – guidance for researchers"

Copy to: Dr Christopher McAloon

Mrs Ceri Jones, University Hospitals Coventry & Warwickshire NHS Trust

Isabella Petrie

Amendments Summary

Three substantial amendments were sent to the REC committee in April, July 2014 and May 2015. The first amendment requested local laboratory blood tests at follow-up assessments. This routinely occurred in normal clinical practice, but formal adoption in protocol was required. The second amendment updated the eligibility criteria to be better aligned with the recently published NICE June 2014 CRT implantation guidelines, which had broadened the national criteria for patient selection [TA120].[17] The July 2014 amendment included a sub-set study on body composition assessment and CS sampling, reflecting recent changes in the literature. More discussion on these sub-set studies can be found later in this chapter. MACE observation extension was requested for 12 months, given the likelihood of a low number of outcome events within the study population in six months. The alteration to eligibility criteria was essential to reflect the real world cohort we hoped to create. The additional changes did not affect study design or have any undue bias upon the cohort study. The final amendment allowed for a blinded intermittent biomarker analysis; however this was actually never undertaken.

Four minor amendments were requested in September 2015, October 2015, February 2016 and March 2016 regarding clarification of definitions in the protocol, statistical analysis, and adding metabolic biomarkers and specifically hs-TnT to the analysis respectively. All these respective amendments were approved by the REC. None of these amendments altered the study design and were performed before outcome allocation and analysis. HS-Troponin was requested to strengthen translational assessment of cardiac source of biomarkers; it did not form part of the prediction of outcome models. All amendments were granted permission by the UHCW Research, Development and Innovation department.

APPENDIX F

Clinical Trials Registration Form

https://clinicaltrials.gov/ct2/show/NCT02541773?term=COVERT&rank=11

Novel Circulating biomarkers Behaviour and Clinical Value in Heart Failure and CRT (COVERT-HF)

Verified by Dr Christopher McAloon, University Hospitals Coventry and Warwickshire NHS Trust, September 2015

Sponsor:	University Hospitals Coventry and Warwickshire NHS Trust
Collaborators:	Medtronic
Information provided by (Responsible Party):	Dr Christopher McAloon, University Hospitals Coventry and Warwickshire NHS Trust
ClinicalTrials.gov Identifier:	NCT02541773

Purpose

The purpose of this study is to understand the behaviour of certain blood markers in patients with heart failure who undergo a cardiac device implantation procedure called cardiac resynchronization therapy (CRT). CRT is an effective treatment for heart failure, but up to 30% of people do not respond and have poor outcomes (1,2). Despite extensive investigation, identifying these patients continues to be a challenge. The study intends to describe the changes in these blood markers before and after CRT and to examine any potential clinical value.

APPENDIX G

Patient Information Sheet

The Role of Circulating biomarkers in Heart Failure patients undergoing CRT

(The characterisation of circulating biomarkers before and after cardiac resynchronisation therapy in patients with heart failure and their role in predicting response)

Summary Page

(For more detailed information, please see the following pages)

You are invited to take part in a clinical research study that is conducted at the University Hospital, Coventry and Warwickshire (UHCW). The study is organised by the University of Warwick (Warwick Medical School) and is funded by research grants from the UHCW Research and Development and the company Medtronic Ltd. It is important that you understand why this research is being carried out and what it will involve for you. A member of the study research team will go through the Information Sheet with you and answer any questions that you have. Please take the time to read the following information carefully, and discuss it with your GP and others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. Your participation in this study is entirely voluntary. Please start by reading the study summary pages. If you think that you might be interested in taking part in this study, then please go on to read the remainder of this Information Sheet.

Part 1 of this Information Sheet explains the purpose of this research study and describes what will happen to you if you take part. Part 2 gives you further detailed information about the conduct of the study.

If you decide to take part, you would be free to withdraw from the study at any time without giving a reason. If you decide not to take part, your treatment and Device implant will not be affected in any way by your decision.

Summary Page

(For more detailed information, please see the following pages)

The decision has been made by your cardiology team that you will benefit from a special heart failure pacemaker. This is called a biventricular pacemaker or '*cardiac resynchronisation therapy*' (CRT). This CRT is used in patients whom have heart failure that meet certain criteria outlined by the health organisation 'National Institute of Clinical Evidence' or NICE. Current evidence suggests that some patients with heart failure will benefit from CRT. The decision has been made that you will benefit from this procedure. Despite strong evidence that people should get better after the procedure, about 30% of people do not improve. The reasons for this are not clear.

Our study plans to see if we can predict those people who will not respond. Recently, new blood markers (circulating biomarkers) have been shown to be different in certain heart conditions, including heart failure. We believe these blood markers may differ in patients that respond and those that do not to CRT. There is currently very little evidence in this particular area of heart failure.

We would like to follow you up before and after the insertion of your CRT. There will be an initial assessment using a short questionnaire about your symptoms and medical history. A heart scan (echocardiogram) will be performed (you will already have had one of these performed). We will perform a walking test which will involve you walking for a few minutes and measuring the distance you can walk. A heart trace (ECG) will be performed as you would normally have at a routine hospital visit. A simple assessment of body fat content (body composition) will be performed using a machine you sit in for two minutes. Finally, we would like to take several blood samples to look at routine blood tests and circulating biomarkers.

After the CRT is inserted we would like to perform two other check-ups at roughly 2 and 6 months after the CRT is implanted. These check-ups would involve exactly the same assessments as your initial assessment. These will be planned to occur at the same time as your routine assessments at UHCW. We would like to take some information about the CRT at the time of implantation and collect some blood when the device is implanted. Finally we would like to review your clinical records over the year after the implant to see what happens to you.

Part 1

Part 1 of this Information Sheet explains the purpose of this study and describes what will happen to you if you take part.

Part 2 gives you further detailed information about the conduct of the study.

What is the purpose of the study?

The purpose of the study is to examine your response to the CRT you will have implanted by the cardiology team. By examining your response through our assessments and scans we want to assess if this can be predicted by new blood markers (circulating biomarkers).

Why have I been chosen?

You have been chosen because the doctors feel that your heart failure may be improved with the CRT implantation. All patients having one put in at UHCW may be asked to join the study.

Do I have to take part?

It is entirely up to you whether or not you take part in this research study. If you decide not to take part, you do not have to give a reason. A decision not to take part in this study will not affect in anyway the standard of care that you receive either at our hospital or from your GP. You will be asked to sign the attached consent form only when you are satisfied that you have been given enough information both verbally and in writing about the study and have decided that you want to participate. You will be given a copy of this patient information sheet and the signed consent form. You should not participate in any other research studies where you are given an experimental treatment or procedure, for the period of this study.

Can I change my mind about taking part?

You are free to withdraw from the study at any time, including after having signed the consent form. This will not impact your normal follow-up after having had a pacemaker implanted.

What will happen to me if I take part?

If you decide to take part in this study, you will have an initial assessment before the pacemaker is implanted and then follow-up for six months after the device is implanted. There will be two further visits to hospital for assessments and scans to try and coincide with your normal follow ups. The follow-up assessments take place at approximately two and six months. Finally we would like to record anything that happens to you in the year after the CRT is implanted by looking at your medical records and providing you with a follow-up phone call a year after your implant.

Study Schedule

Recruitment: You receive your '*patient information sheet*' and will be asked to sign a consent form to participate in the study after you have read the information and had time to think it through. You will only be asked to sign the consent form when you are happy all your questions have been answered.

First Assessment (morning of implant): Clinical assessment, quality of life questionnaire, echocardiogram, ECG, simple walking test, body composition assessment, and blood samples.

Pacemaker device Implantation: Information about the pacemaker and procedure are recorded. Blood samples during the procedure will be taken (explained later).

Two and six months follow-up after implant (days of pacemaker check): Clinical assessment, quality of life questionnaire, echocardiogram, simple walking test, body composition assessment, and taking blood samples

What do the assessments involve?

Clinical Assessment: Questionnaire about symptoms and medical history

Quality of Life Questionnaire: Standardised questionnaire to assess quality of life

Echocardiogram: An ultrasound scan of the heart (you should have had this done before)

ECG: A heart trace (you would normally have this done on routine hospital visits)

Walking Test: A walking test to see how far you can walk on the flat in six minutes

Body Composition Assessment: A machine that measures for body composition (muscle and fat content) by sitting in a chamber for two minutes.

Blood Samples: Several blood samples will be taken with one blood test and this will be repeated at all three assessment periods. Some of these blood samples will be sent to our local laboratory for routine blood tests on kidney function, full blood count, long term glucose control (HBA1c) and Brain Naturetic Peptide (Blood test used to look for heart failure). The remaining blood samples will be stored to test for these circulating biomarkers.

Procedure Blood Samples: During a routine CRT procedure a tube is placed into a vein in the heart to help insert one of your leads into the heart. This is all routine as part of the procedure. We would like to take some blood from that vein via the tube to look at circulating biomarkers directly coming from the heart.

What do I have to do?

During the study you will need to follow the instructions of your study doctor. It is very important that you attend all scheduled visits, so please let your study doctor know if this is not possible.

What does body composition (BodPod) assessment involve?

The BodPod is a very accurate method of measuring your body composition. It does this by calculating your weight and volume (measured by air displacement) to determine body density. Your body fat percentage is determined from density and other measurements.

You will first be asked to change into close fitting underwear. There is a screen and a couch provided to change in private. Once you are changed you will be asked to stand on a set of weighing scales for a few seconds. We will then get you to sit in the BodPod (a chamber to measure air displacement), where we will ask you to sit still for two minutes while the measurements are done.

Is Bod Pod safe?

Yes Bod Pod is very safe. An experienced operator will be present throughout the measurements. There is an emergency button if you want the door to open at any time.

What are the possible inconveniences and risks of taking part?

There is little risk with the walk test because you are briefly exerting yourself. This test is performed routinely in heart and lung patients to assess exercise capacity. You can stop at any point and a cardiology doctor will be present throughout the test.

It is possible that some discomfort or bruising may occur when blood samples are taken. However, all care will be taken to minimise this.

Taking blood during the procedure carries no risk at all to you during the procedure.

What are the possible benefits?

To be a part of the assessment of a new blood test to see if they will help identify those patients that will definitely benefit from CRT insertion. It may also help other patients avoid an unnecessary procedure (despite it having a low complication rate).

What happens when the research study stops?

Once you have completed the follow-up for six months no further research appointments will be made. We will continue for the next six months (months 6-12 after CRT implantation) to record anything that happens to you. We will do this by reviewing your medical records and we may phone you at 12 months. You will continue with routine follow-up for the CRT after the study finishes.

What happens if you withdraw from the study before the end of the follow-up period?

You would be free to withdraw from the study at any time without giving a reason and a decision to withdraw from this study will not affect in any way the standard of care that you receive either at our hospital or at your GP. If you decide to no longer take part in the study, we will ask you if we can keep the information (including blood) you have already provided us. However, if you want all information (including blood) removed from storage we will delete it all at your request.

What if there is a problem?

Any complaint about the way you have been dealt with during the study or any possible harm you might have suffer will be addressed. The detailed information on this is given in Part 2 of this Information Sheet.

Will my taking part in the study be kept confidential?

Yes. All information about your participation in this study will be kept strictly confidential. The details are included in Part 2 of this Information Sheet.

Contact Details:

If you have any questions regarding your rights as a study participant or about the way this study is conducted please contact your study doctor or nurse. They will be happy to answer them.

Name: Dr Christopher McAloon/ Dr Faizel Osman

Address: University Hospitals Coventry and Warwickshire (UHCW) NHS Trust,

Clifford Bridge Road, Coventry, CV2 2DX

Tel.: 02476 96 5813

This completes Part 1 of the Information Sheet. If the information in Part 1 has interested you and you are considering participation, please continue to read the additional information in Part 2 before making any decision.

Part 2

What if new information becomes available?

Sometimes during the course of a clinical study, new information may become available about blood markers and so the study procedures may be changed or a new analysis may be planned. If any of this happens your study doctor will inform you about it, answer any questions you may have and discuss whether or not you want to continue in the study. If you decide to withdraw from the study, your CRT follow-up will continue as normal. If you decide to continue in the study, you will be asked to sign an updated consent form containing the new information.

At any point during the study you can ask your study doctor for the results of the tests that have under taken.

What will happen if I don't want to carry on with the study?

You are free to withdraw your consent to participate in this study at any time and without giving a reason. This will not affect in any way the standard of care you receive. No further assessments in the study will be arranged but follow-up for your pacemaker will continue as normal. We will ask you if the information and samples you have already provided can still be used in the study. You are entitled to request the destruction of your laboratory samples and that no further laboratory samples are taken.

What if there is a problem?

Complaints:

If you have a concern about any aspect of this study, you should ask to speak to your study doctor or nurse who will do their best to answer your questions (see Part 1 above for contact details). If you remain unhappy, you can contact the UHCW Patient Advice Liaison Service or 'PALS'

Patient Advice Liaison Service

University Hospitals Coventry and Warwickshire NHS Trust

Clifford Bridge Road

Coventry

CV2 2DX

Telephone: 0800 028 4203

Will my taking part in the study be kept confidential?

All information about you will be kept and handled in a strictly confidential manner, in accordance with applicable laws and/or regulations. All study participants will be given coded study numbers, and no names will be used for the storage of information and samples. Any information that leaves the hospital will not have any identifiable personal data attached to it. All information collected during the course of the study will be accessible only to the researchers participating in the study and will be kept on hospital/university computers that can only be accessed by the study researchers using a password. By signing the study consent form you consent to the collection and use of your personal data for the study. This may include sensitive personal data about your age, ethnic origin and health. You may withdraw your consent at any time. If you consent to take part in this study, your medical records will be accessed by members of the study team for the purpose of checking study procedures and data. Your medical records may also be looked at by representatives from national and international regulatory authorities to check that the study is being or has been carried out properly. However, your name and identity will not be recorded or disclosed outside the study clinic.

Your study doctor is responsible for the code list by which you can be identified. The code list may be reviewed by representatives from regulatory authorities, but it will not be disclosed outside the study clinic. Apart from this, the information about you will be given the usual confidentiality that all National Health Service patients can expect.

What will the results of the study be used for?

The results of the study may be published in medical literature, but you will not be identified and the confidentiality of all study participants will be respected. You have the right to request information about your study data and to be provided with a copy of them.

You also have the right to request that any inaccuracies in such data be corrected. If you wish to make a request, then please contact Dr. Osman at UHCW. If there is important new information about the study we will inform you.

The study data will also form the basis of the lead investigators research degree (PhD). The results will be used in the lead investigator's thesis (research project). All information used will be anonymous and kept confidential.

Involvement of the General Practitioner/Family Doctor (GP)

Your study doctor/nurse is obliged to inform your GP and other specialist doctors who are involved in your healthcare about your participation in the study. You will be asked to give your consent for that when you sign the attached consent form.

What will happen to any samples I give?

Some of the blood samples will be tested by our local laboratory for routine tests that the hospital does daily. The likelihood is that you will have had these blood tests performed previously. These samples will be sent to the laboratory and be tested within 24 hours. The routine blood tests have to be performed at a set time so the results can be compared to the other information that have been recorded for the research at that time.

The other blood samples will be stored at the University Hospitals of Coventry and Warwickshire and at the University of Warwick and analysed for the research purposes of this study. We hope that there will be some samples left over and we hope to store these for future research relating to heart failure. However, you may say that you only want your samples used for this study and no others, therefore any samples not used will be destroyed (you are expressly asked about this in the consent form).

Some of the samples will be sent to Kings College London for a specific test that we want to perform as part of the study. Unfortunately, we are unable to perform this test at UHCW or the University of Warwick. These samples will be transferred securely to Kings College London. Each sample will have a unique identifying number and no personal details attached to them.

All samples will be coded to make sure that your identity remains confidential. The results of these research investigations are unlikely to have any implications for you personally.

If you withdraw from the study at any time, you are entitled to request the destruction of your laboratory samples and that no further laboratory samples are taken.

Will I get the results of my routine blood tests?

The routine blood tests will be sent to the principle investigation and recorded in the research notes. The results will be given to you when they are available.

Will I get the results of my echocardiogram?

The images taken at the time of the assessment need to be assessed by the investigator and this can take a short time. The results will not be immediately available, but if requested they will be available. A formal report will go on to your electronic clinical record which is available to any doctor or nurse.

What happens if there is an abnormal result?

Firstly the results will be given to you by a clinician (likely the lead investigator). If required these results will be sent to the Cardiology consultant looking after your care. Most likely any abnormal results will not be serious and can be fed back to your general practitioner to be actioned. In the unlikely event that a serious problem is identified a management plan will be implemented at the time to remedy the problem, which may involve the hospital team.

Will any genetic tests be done?

No genetic tests will be performed within this study.

What will happen to the results of the research study?

Once the study is complete the results will be published and a final report will be written. Information relating to the study may be communicated in scientific meetings. You will not be identified in any reports or publications.

Who is organising and funding the research?

You are invited to take part in a clinical research study that is conducted at the University Hospitals of Coventry and Warwickshire (UHCW). The study is organised by the UHCW and University of Warwick (Warwick Medical School) and is funded by UHCW research, development and innovation department and the device company Medtronic Ltd. The doctors and nurses responsible for your treatment will not receive any personal payments.

Will I receive any expenses for taking part in this study?

Unfortunately there will be no money available for expenses (travel costs etc). To limit this impact we are attempting to perform all assessments at your routine hospital appointments. For instance we will do your initial assessment on the morning of your implant. On occasions, it might not be possible to do your assessment on the day of your routine appointment and you will be invited to attend at another occasion (at your convenience).

Who has reviewed this study?

Before any research is carried out, it has to be thoroughly checked by an ethics committee. The committee makes sure that the research is appropriate to do in accordance to Good Clinical Practice (GCP) principles, regulations and guidelines. This study has been reviewed and approved by the local Research Ethics Committee which is entirely independent of any hospital trust.

If you wish to take part in the study you will be asked to sign the consent form overleaf. A copy of your signed consent form and this patient information sheet will be given to you to keep.

Thank you for taking the time to read this information sheet.

APPENDIX H

Concent Form

Patient ID Number: _____

Trial: The Role of Circulating biomarkers in Heart Failure Patients Undergoing CRT

Chief investigator: Dr Faizel Osman

Please Initial box

1- I confirm that I have read and understand the information sheet dated 14th July 2014 for the above study. I have been given sufficient time to consider the information and to seek other advice. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2- I understand that I am being invited to take part in a research study. I am not taking part in any other clinical trial at this time. I understand the risks and benefits and I freely give my informed consent to participate in the research study described in the information sheet, under the conditions stated in it. ☐

3- I understand that my participation is voluntary and that I am free to withdraw at anytime, without giving any reason, without my medical care or legal rights being affected. ☐

4- I understand that relevant sections of any of my medical notes and data collected during the study may be looked at by the study researchers where it is relevant to my taking part in this study. ☐

5- I give consent that my GP and specialist doctors involved in my healthcare may be contacted and access given to my medical notes held by my GP and at other hospitals. ☐

6- I am aware that I will receive a signed copy of this Patient Information Sheet and Informed Consent Form, which is mine to keep. ☐

7- I agree to donate blood samples for this study. I understand that samples will be stored at UHCW and the University of Warwick and analysed for the research purposes of this study and for future research, with relevant ethical approval, relating to heart failure. I understand that the results of these research investigations are unlikely to have any implication for me personally. ☐

8- I agree to my samples that are not being used in this study to be kept by Warwick Medical School and Kings College London for future analysis, in projects related to heart failure. (This is optional and samples will be destroyed if you do not want them kept in storage). ☐

Please tick the box to tell us your decision

Yes ☐ **No** ☐

9- I agree to take part in the above study. ☐

Name of person giving consent Date Signature

…….. …….. ……

APPENDIX I

Research Protocol

The Characterisation of Circulating biomarkers before and after Cardiac Resynchronisation Therapy in patients with Chronic Heart Failure and their Role in Predicting Response

Version 11 and 8th March 2016

STUDY SUMMARY

TITLE

The Characterisation of Circulating biomarkers before and after Cardiac Resynchronisation Therapy in patients with CHF and their role in predicting response.

DESIGN

A prospective, non-randomised, self-control trial examining novel circulating biomarkers in CHF patients undergoing CRT implantation.

AIMS

To evaluate whether novel vascular markers [ECM and miRNA] maybe predictive of CRT response and major adverse cardiovascular outcomes. Furthermore to characterise the behaviour of these novel circulating biomarkers and body composition in CHF patients before and after CRT implantation and examine their potential clinical value.

OUTCOME MEASURES

Primary outcome measure is clinical response to CRT implantation. The secondary outcomes are major adverse cardiovascular events (mortality and heart failure hospitalisation).

POPULATION

Local population of Coventry and Warwickshire with chronic heart failure selected to undergo CRT implantation.

ELIGIBILITY

All patients being implanted for CRT who meet NICE criteria(specific criteria below).

DURATION

Two and a half years.

1. INTRODUCTION

1.1 BACKGROUND

CHF is a common, costly and disabling condition affecting almost 1 million people in the UK[2]. CRT is one of the most effective heart failure therapies to emerge in the last 25 years and is applicable to a third of all symptomatic heart failure patients[234]. It involves implantation of pacemaker leads to pace the right atrium, right ventricle and left ventricle (via the coronary sinus) to resynchronise cardiac contraction. Several prospective randomised studies have shown that CRT is associated with a significant reduction in hospitalization rates for heart failure and improved long-term survival [22,23,28].Consequently, CRT has gained widespread acceptance as a safe and efficacious therapeutic strategy.

Recently indications for suitable patients have been broadened [17] based on more focused evidence of patients that may benefit from CRT implantation [38,319]. The new guidance requires a left ventricular ejection fraction ≤35%, optimal medical therapy, in either sinus rhythm or atrial fibrillation (where satisfactory level of biventricular pacing can be achieved). All NYHA symptoms classification can be considered; I (QRS>150msec on resting ECG), II/III/IV (QRS>150msec or 120-149msec with LBBB)[17,47,56]. Patients undergoing pacemaker implantation or upgrade should be considered for CRT if LVEF <35% and likely to be pacing dependent >40% of the time[47]. However, despite these indications, a significant proportion of patients (approximately 20-30%) remain unresponsive and have recurrent hospitalisations for heart failure with no improvement or even deterioration in symptoms with CRT pacing[23,27,28,31,41,250,319]. As such, better identification of suitable patients would be of great benefit to the NHS.

Novel circulating biomarkers of CHF (miRNA, ECM remodelling markers and GDF 15) have demonstrated that their expression is altered on implantation of CRT and alters significantly between responders and non-responders[112,119,120,126,197]. MiRNA are small non-coding RNAs that modify gene expression at the post-transcriptional level and have emerged as key regulators of cardiac growth, vascular development, and angiogenesis[191,276]. Altered levels have been reported in patients with heart failure, coronary artery disease, and diabetes[155,188,197]. Recently miRNA has been profiled in CHF patients undergoing CRT implantation and demonstrated that there is alteration in expression between responders and non-responders in the peripheral blood samples[197]. Only a small number of miRNA were

examined and potentially these markers offer very stable and durable biomarkers of CRT response [164,197].

Extracellular matrix remodelling is implicated in myocardial fibrosis, which directly correlated with left ventricular adverse remodelling and ultimately adverse outcomes [119]. Controversy on the response of specific ECM markers exists and exactly how they alter in responders [119,126]. Clarification is required to delineate their role and whether they are potential predictors of response and adverse outcomes.

GDF 15 is a marker of myocardial stress demonstrated to associated with heart failure and can be used for risk stratification[325]. Recently higher levels of GDF15 prior to CRT have been shown to predict non-response and higher occurrence of adverse outcomes [112]. The levels of GDF 15 correlate with the behaviour of brain naturetic peptide (BNP) and together they are synergistic for predicting response and outcome [112].

Profiling novel circulating biomarkers in CHF demonstrated the alterations that occur in the body as a result of adverse remodelling of the ventricles. The common pathway for the deterioration in CHF from a given aetiology is the activation of the neurohormonal pathway, inflammation, extracellular matrix remodelling and ultimately cardiac fibrosis. Biomarkers allow this process and the particular stage to be identified. Endothelial dysfunction and body composition alterations are hallmarks of this adverse remodelling systemically. Cardiac cachexia (defined as >5% non-oedematous weight reduction over six months) is directly linked to adverse outcome and inversely linked to BNP [206,214]. This allows a systemic measure of the deconditioning that the body goes through with adverse remodelling [208]. Certain biomarkers may have a role in this that has not yet been linked. CRT moreover offers the opportunity to examine what happens in reverse remodelling and whether there is a link to potential novel circulating biomarkers.

A robust examination of the selected biomarker profiles requires a precise cardiac measure of their expression. Blood samples from the coronary sinus allow a direct measure of biomarker release from the heart to be measured [263,326]. The profiling of biomarkers [miRNA, GDF 15, and ECM] directly from the heart and comparing them to systemic blood allows differences to be identified and explored.

Characterisation and profiling of these novel biomarkers in CHF, before and after implantation, is important and could help clarify their role in identifying those who may

benefit from such therapy. Understanding biomarker behaviour in the heart and in the periphery will allow the robustness of these markers to be challenged. Charting these markers with important correlation to echocardiographic and body composition markers will challenge the real valve of these markers and their behaviour. This would enable effective use of limited health-care resources, especially in the current financial climate, to target those most likely to benefit and have an enormous clinical impact. This project will generate novel pilot data for a competitive research grant application at the end of the study.

The study will evaluate circulating biomarkers in heart failure patients before and after CRT, in collaboration with Dr Harpal Randeva (University of Warwick) and Professor Manuel Mayr (Kings College London).

1.2 RATIONALE FOR CURRENT STUDY

The study rationale is to determine the clinical and predictive value of novel circulating biomarkers (ECM, miRNA) in determining CRT response (clinical and echocardiographic) and major adverse cardiovascular outcomes. The further rationale is to characterise behaviour of specific novel circulating biomarkers on implantation of CRT devices.

1.3HYPOTHESIS

'The profiling and characterisation of novel circulating biomarkers (miRNA, ECM markers, GDF15) in CHF patients undergoing CRT implantation will provide potential indicators of patient response and major adverse cardiovascular outcomes'

2. STUDY OBJECTIVES

2.1 PRIMARY OBJECTIVE

To examine if novel circulating biomarkers [GDF-15, ECM remodelling markers, miRNA] may predict response and major adverse cardiovascular outcome's in CHF patients undergoing CRT implantation

2.2 SECONDARY OBJECTIVE

1. To characterise novel biomarkers in CHF patients undergoing CRT.
2. To characterise miRNA expression in patients with severe left ventricular systolic dysfunction.
3. To correlate CRT response and body composition alterations
    a) To correlate novel circulating biomarker behaviour and body composition changes in CRT responders and non-responders
4. To compare novel circulating biomarker expression systemically (peripheral blood) and from the heart (coronary sinus). Does the alteration in expression delineate the role of these novel biomarkers in adverse remodelling of the heart?

3.1 STUDY DESIGN

3.1 PARTICIPANTS SELECTION

A prospective, non-randomised, self-control study of unselected heart failure patients undergoing CRT implantation, all recruited within two years (~82 CRT implanted 2012 at UHCW). All participants having CRT implantation at UHCW will be screened using the eligibility criteria (section 4).

Participants will have three assessments within six months (baseline, 6 weeks and 6 months). All visits coincide with routine clinical visits for CRT implantation and interrogation. Assessments at all three time points will include clinical data (including NYHA functional class), quality of life measurements (Minnesota Living with Heart Failure questionnaire), echocardiography data (left ventricular volumetric assessment, ejection fraction, dyssynchrony measurements), electrocardiograph, functional capacity (6 minute walk test) and body composition assessment (air displacement). Peripheral blood samples will be taken to examine novel circulating biomarkers and to examine renal function, full blood count, diabetic control (HBA1c – only diabetics) and heart failure control (Brain Naturetic Peptide).

Aetiology of heart failure will be defined as ischaemic or non-ischaemic cardiomyopathy. Ischaemic cardiomyopathy will be defined with either previous myocardial infarction, previous coronary bypass grafting, significant ischemic disease with previously treated stenoses of ≥50% of lumen diameter in ≥1 major epicardial coronary artery and cardiac MRI

defining ischaemic aetiology. Diabetes will be defined as either patients on therapy [anti-diabetic therapy or insulin], a random venous plasma glucose concentration > 11.1 mmol/l, a fasting plasma glucose concentration > 7.0 mmol/l (whole blood > 6.1mmol/l), a two hour plasma glucose concentration > 11.1 mmol/l two hours after 75g anhydrous glucose in an oral glucose tolerance test (OGTT) or HbA1c of 48 mmol/mol (6.5%) is recommended as the cut point for diagnosing diabetes [324].

3.2 BLOOD SAMPLING AND LABORATORY ANALYSIS

Participants are asked to starve for two hours and rest for one hour before blood sampling. Coronary sinus blood sampling occurs at the time of coronary sinus cannulation before contrast is injected. Blood samples (serum/plasma) are taken in citrate and EDTA
tubes. The samples stand at room temperature for a minimum of 30 minutes and undergo centrifugation within an hour. Centrifugation (3500rpm for 10 minutes) occurs at room temperature. Samples are then stored in -80 freezer.

Samples will undergo final analysis at the University of Warwick (Dr Harpel Randeva laboratory) and Kings College London (Professor Manual Myer). Novel circulating biomarkers of heart failure that exist as proteins in the serum will analysed using ELISA techniques previously outlined in publications [119,120,126,318]. The specific novel circulating biomarkers represent different aspects of adverse ventricular remodelling; myocardial stress (GDF-15) and ECM remodelling (MMP-2,-9, PINP, PIIINP, ICTP). These markers will undergo level quantification using ELISA techniques that have previously described in the literature [131,265]. MiRNA profiling will be undertaken in coronary sinus and peripheral samples and will be compared to those taken after CRT implantation. Responders will be compared to non-responders to determine variation in profile between these two distinct groups. Profiling will be performed by extraction of RNA amplifying it and quantifying by polymerase chain reaction as outlined in previous publications [147,276]. High-sensitive-Troponin is a specific biomarker produced in the heart and it will be utilised to examine the production source (cardiac vs peripheral) of the respective biomarkers outlined above.

Participants that have undergone body composition assessment will have blood samples assessed for metabolic and insulin related markers to explore the relationship between heart

failure and the body's metabolism. Quantification of these markers will further strengthen the secondary aim of understanding the physiological changes to the body in heart failure patients and how these alter with CRT placement.

3.3 DEVICE IMPLANTATION

CRT devices (pacemaker of defibrillator) are implanted by two independent operators at our single centre in a standard fashion. CRT is implanted traditionally in the left deltropectroal groove. Venous access is via the cephalic>axillary>subclavian veins (all operators can cannulate either vein based on individual patient). Right ventricular lead for the majority of patients will be implanted at the right ventricular apex. Right atrial leads will be planned to be implanted in the right atrial appendage. Patients in permanent Atrial Fibrillation will not have a right atrial lead implanted. Coronary sinus will be cannulated and angiography performed to roadmap anatomy for lead deployment site. The most lateral position will be favoured in a basal/ mid-cavity position. At the point of coronary sinus cannulation blood samples (plasma/serum will be taken). Post procedure patients undergo targeted echocardiography and a chest x-ray film. Post procedure on the day of implant patients will undergo CRT interrogation and optimisation of programming.

3.4 ECHOCARDIOGRAPHY

A full standard echocardiographic examination will be performed at all three assessments including grey-scale images optimised for 2D and colour tissue doppler imaging (TDI) from the 3 standard apical views (2, 3, 4 chamber). All echocardiograms will be performed on the same machine by the same operator for each participant in line with current echocardiographic guidelines [327]. The left ventricular end systolic and diastolic volume will be measured and LVEF will be calculated using the Simpson's biplane method.

3.5 INTER- AND INTRA-OBSERVER VARIABILITY ECHOCARDIOGRAPHY STUDY

An inter- and intra-observer variability study will be performed to ensure standardisation of echocardiographic examinations. Twenty percent of echocardiograms will be randomly selected to have measurements and conclusions reviewed. An independent cardiologist/ cardiac physiologist (accredited by the *British Society of Echocardiography)* will be blinded to

selected echocardiograms and will validate reporting and measurements of these examinations.

3.6 BODY COMPOSITION ASSESSMENT

Air-displacement plethysmography (Bod Pod) is an easily accessible and safe tool to measure body composition [261]. The assessment is reproducible and is comparable to other measures of body composition [261]. The major advantage of Bod Pod is that it is safe for patients who have had a CRT implanted. Bioelectrical impedance another accurate measure of body composition is not recommended by manufacturers of CRT to be used after implantation, despite evidence demonstrating it is safe [260]. The University of Warwick has one of the few facilities in their Human Metabolic Unit to perform Bod Pod quickly and effectively. The assessment will take place at all time points and only require a few minutes to get a full set or results.

3.7 DEFINITION OF RESPONSE

The criteria for CRT clinical and echocardiographic response will be applied short-term [six week review] and long-term [six months]. Overall response will be determined on the final review. The clinical definition of CRT response will be a two out of three criteria [↓≥1 NYHA, ↑≥10% 6MWT distance, ↓MLHFQ score>5] (Table One). Echocardiographic response will be defined as ≥15% reduction in LVESV. Continous echocardiogram data will be correlated with novel circulating biomarkers and body composition variables.

Participants will be defined as a non-responder if during the six months follow-up they die or undergo a heart transplantation. Hospitalisation will not influence response criteria.

Table 1. Clinical and echocardiographic response criteria

Clinical Response at Six Months

Two out of Three:
↓≥ 1 NYHA
↓MLHFQ score > 5
↑≥ 10% 6MWT distance

Echocardiographic Response at Six Months
↓≥ 15% LVESV

↓= reduction, ↑= increase

3.8 MAJOR ADVERSE CARDIOVASCULAR OUTCOMES

MACE will be calculated as a composite [mortality and first heart failure hospital admission] over a 12 month period from CRT implantation. Individual event rates for mortality and heart failure admissions will be calculated. Heart failure hospital admissions will be defined as overnight stays with intravenous diuretics treatment.

3.9. STUDY OUTCOME

The primary outcome for the study will be clinical response at 6 months. The secondary outcomes for the study will be echocardiographic response and MACE.

4. PARTICIPANT ENTRY

4.1 PRE-REGISTRATION EVALUATIONS

Participants must have clinical investigations to allow assessment under the clinical criteria of the NICE guidance for CRT implantation. Left ventricular ejection fraction can be originally evaluated by echocardiography, cardiac magnetic resonance imaging and nuclear

myocardial perfusion scanning (this does not replace baseline echocardiographic evaluation).

4.2 INCLUSION CRITERIA

7. Age > 18 years
8. Left ventricular ejection fraction ≤35% on echocardiography
9. NYHA Class III/IV symptoms or milder symptoms with:
    c) NYHA I (LVEF <35% and QRS>150msec on resting ECG)
    d) NYHA II (LVEF <35% with either QRS>150msec or QRS 120-149msec with Left Bundle Branch Block on resting ECG)
10. Optimal medical therapy for heart failure that the patient tolerates (ACEi, Beta-Blocker, Mineralocorticoid) for > 3 months
11. QRS duration ≥120-149msec with LBBB on resting ECG **or** QRS duration >150msec on resting ECG
12. Patient consent to participation in the study

4.3 EXCLUSION CRITERIA

6. Acute heart failure decompensation < 6/52 before implant
7. Significant cognitive impairment
8. Acute coronary syndrome < 6/52 before implant
9. Chronic kidney disease stage V (requiring dialysis)
10. Terminal illness with likely survival < 1 year after implant

4.4 PROCEDURE AND POST PROCEDURE STUDY EXCLUSIONS

3. Failure of procedure (e.g. coronary sinus anatomy)
4. Complication resulting in poor/ none biventricular pacing (e.g. phrenic nerve stimulation, lead displacement/ damage)

4.5 ECHOCARDIOGRAPHY

Imaging quality is essential for the study to determine volumetric assessments and perform Simpsons biplane assessment for left ventricular ejection fraction. Biplane assessment will be the standard, but single plane assessment will be accepted dependent on image quality. Participants that have poor overall images (e.g. High Body Mass Index) then patients will be excluded from echocardiographic response analysis but included in clinical response and major adverse cardiovascular outcome assessment.

All participant images will be stored on the local institution echocardiography database and available to all users. All images will be subject to inter- and intra-observer validation study. Twenty percent of all images will be reviewed by an independent cardiologist /cardiac physiologist blinded to patient outcomes.

5. ADVERSE EVENTS

This is an observational study, without intervention. Definitions of adverse and serious adverse events are unlikely to have a need to be applied. Clinical events in a CHF population undergoing CRT implantation are expected in the natural history of the period of observation. Only directly attributable events to the study will be logged as adverse events and will be graded depending upon situation. Specifically hospital admissions or deaths related to heart failure or another cardiac cause will not be graded as an adverse event. Procedural issues (including complications) will not be designated at adverse events as the study does not influence the decision to implant CRT or the procedure itself. All clinical events will be logged and if it meets MACE criteria will be logged as a MACE event.

5.1 DEFINITIONS

Adverse Event (AE): any untoward medical occurrence in a patient or clinical study subject.

Serious Adverse Event (SAE): any untoward and unexpected medical occurrence or effect that:

1. **Results in death**
2. **Is life-threatening** – *refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have*

caused death if it were more severe

3. **Requires hospitalisation, or prolongation of existing inpatients' hospitalisation**
4. **Results in persistent or significant disability or incapacity**
5. **Is a congenital anomaly or birth defect**

Medical judgement should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

5.3 REPORTING PROCEDURES

All adverse events will be reported.

5.3.1 Non serious AEs

All such events, whether expected or not, will be recorded.

5.3.2 Serious AEs

An SAE form will be completed and faxed to the Chief Investigator within 24 hours. However, relapse and death due to heart failure and hospitalisations for elective treatment of a pre-existing condition do not need reporting as SAEs. Complications from the CRT implant does not need to be reported as SAE. It is anticipated that there will be no SAE's as there is no intervention being undertaken directly by the study.

Reports of related and unexpected SAEs should be submitted within 15 days of the Chief Investigator becoming aware of the event, using the NRES SAE form for non-IMP studies.

Local investigators will report any SAEs as required by their Local Research Ethics Committee, Sponsor and/or Research, Development & Innovation Office.

ASSESSMENT AND FOLLOW-UP

Patients will be screened once CRT implantation decision is made, based on national guidelines (eligibility criteria above). Screening will be performed in patients attending weekly arrhythmia clinic (main portal for CRT referral to UHCW). Ward based patients may be listed directly for CRT implantation and will be screened. These patients need to be well before enough for elective (non-urgent) implantation (as per eligibility criteria). A local district general hospital (George Eliot, Nuneaton) refers patients directly for CRT implantation and these patients will be screened once a decision is made to implant. Patients screened and who meet eligibility criteria will have the study mentioned to them by the clinician making the decision to implant the CRT. Each patient undergoing elective CRT implantation attends a pre-operative clinic (minimum 3 days before procedure) the researcher will approach the patient and provide the patient information sheet at this attendance. A follow-up phone call will be offered to clarify any outstanding questions. Consent will be obtained on the morning of implantation (before assessment). In the rare situation that a patient attends as an unplanned (non-urgent e.g. due to cancellation), the patient will be given the patient information sheet or asked if it can be posted to them a minimum of 48 hours before the procedure.

At the time of procedure, data will be collected on the device and the implantation. These will be two further follow-up reviews after the device implantation. These will correspond with the CRT follow-up. Approximately these will take place at two and six monthly intervals.

7. STATISTICS AND DATA ANALYSIS

Statistical analysis will be performed with IBM SPSS Statistics 22 (USA) software package. Categorical variables will be reported as numbers and percentages. Comparison analysis for categorical data will be performed using the Chi-Squared test. Where smaller than expected values are derived Fishers Exact test will be performed. Continuous data will undergo histogram plots and Kolmogorov-Smirnov tests of normality. Normally distributed data will be reported as mean ± standard deviation (SD). Non-normally distributed data will be reported as median ± interquartile range (IQR). Comparison analysis for independent and dependent normally distributed continuous data will be performed using unpaired or paired t-tests respectively. Where paired and unpaired continuous data is non-normally distributed a

comparison analysis will be performed using Mann-Whitney U test or Wilcoxon Signed Rank test respectively. ANOVA or Friedman test will be used to perform comparative analysis of multiple continuous data sets with normal and non-normal distribution respectively. A value of $p<0.05$ was used for statistical significance.

Based on response, patients will be categorised as a responder or a non-responder [clinical and echocardiographic]. Univariate analysis will be performed using logistic regression on all individual biomarkers and known predictors of response [LBBB, gender, aetiology, QRS duration]. Multivariate logistic regression analysis will be performed correcting for known cofounders. Survival analysis will be conducted on those free from MACE based on grouping above or below the median individual biomarker level, these groupings will be used to predict risk of MACE.

Sequence of individual biomarker levels will have a t-test performed to assess the difference between baseline and follow-up [six weeks and six months]. A correlation analysis of biomarker levels at baseline and follow-up with CRT response and MACE will be performed. Correlation coefficient will be presented depending on distribution.

Paired t-test will be performed to compare individual biomarker levels in peripheral venous and coronary sinus samples. Receiver operating characteristics (ROC) curves will be created. ROC curve coefficients will be statistically compared for peripheral venous and coronary sinus biomarkers.

Correlation matrix will be performed to compare multiple continuous variables (biomarker levels, echocardiography, clinical, body composition] to explore the possible relationships between variables in responders and non-responders.

8. REGULATORY ISSUES

8.1 ETHICS APPROVAL

Ethical approval for the study was obtained from the South Birmingham Regional Ethics Committee in October 2013. University Hospital Coventry and Warwickshire is the only participating site.

8.2 CONSENT

Consent to enter the study must be sought from each participant only after a full explanation has been given, an information leaflet offered and time allowed for consideration. Signed participant consent should be obtained. The right of the participant to refuse to participate without giving reasons must be respected. After the participant has entered the study the clinician remains free to give alternative treatment to that specified in the protocol at any stage if he/she feels it is in the participant's best interest, but the reasons for doing so should be recorded. In these cases the participants remain within the study for the purposes of follow-up and data analysis. All participants are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment.

8.3 CONFIDENTIALITY

The Chief Investigator will preserve the confidentiality of participants taking part in the study and is registered under the Data Protection Act.

8.4 INDEMNITY

Indemnity for the study is provided by the sponsor the University Hospital of Coventry and Warwickshire.

8.5 SPONSOR

The study is sponsored by the University Hospital of Coventry and Warwickshire.

8.6 FUNDING

The study is funded by the research and development department of the University Hospitals of Coventry and Warwickshire. Further funding has been provided by Medtronic Ltd. This funding is confirmed and in place.

The funding principally funds the wages of the principle investigator and the resources needed for performing the study. Funding will be needed for the analysis of the blood samples to look at the circulating biomarkers including possible purchase of a centrifuge. Agreements are otherwise in place analysis of samples to be done in collaborators laboratories. Patient assessments will be performed using UHCW resources which the principle investigator will perform. Cost basis will be low for delivery of the study. Patients will not be paid for participation or travel as review points are arranged to correspond with routine checks.

8.7 AUDITS AND INSPECTIONS

The study will be subject to inspection and audit by the Sponsor and other regulatory bodies to ensure adherence to GCP.

9. STUDY MANAGEMENT

The day-to-day management of the study will be co-ordinated through UHCW Cardiology Department by Dr Christopher McAloon.

10. PUBLICATION POLICY

The intention for the study is to publish the anonymous results and analysis in a peer review cardiology journal and present the data to international conferences.

APPENDIX J

Clinical and Procedure Data Collected

1.Clinical Baseline Data

The following section outlines the specific clinical data recorded at the baseline study visit.

Baseline Data Collection

Demographic information was collected at the baseline assessment; age, gender, ethnicity, device type. There is overlap with the information captured in study I

Medical History

1. Aetiology Cardiomyopathy
2. Heart Disease of another cause
3. Previous Myocardial Infarction
4. Presence previous Coronary Artery Bypass Grafting
5. History of Hypertension
6. Diabetes Mellitus (Type, Complications)

Other conditions were noted in other comments if observed. These included previous PCI for coronary artery disease, presence of current angina, a history of or current atrial fibrillation and a history of chronic obstructive pulmonary lung disease. Any previously inserted cardiac device (Pacemaker or ICD) was recorded under '*Heart Disease of another cause*'. The specific device was recorded with the number of leads in-situ. If the device was

an ICD it was noted whether any previous therapies (anti-tachycardia pacing and/or delivery of a shock).

Clinical Assessment of Symptoms

During the clinical assessment, data was entered on to a specific form (**Appendix K**). Participants were initially assessed to see if they had HF symptoms. NYHA symptom classification was assessed at each study visit. Specific symptoms and exercise tolerance was assessed at every study visit:

Dyspnoea at rest

1. Dyspnoea with minimal effort
2. Dyspnoea with moderate effort
3. Nocturnal dyspnoea
4. Ankle Oedema
5. Dyspepsia
6. Syncope/Pre-syncope
7. Palpitations
8. Fatigue

Medication History

All medications which the participant was taking at the time of assessment were recorded. The dose, route and frequency of each medication were also recorded. HF medications for addressed in particular:

1. ACEi
2. Angiotensin Receptor Blocker
3. Beta-Blockers
4. MRA
5. Diuretics

The doses, frequency and route were all recorded. Optimal HF and stability of treatment will have already been established in the screening process. Where the above optimal medical treatments for HF were not prescribed, a reason as part of the screening process was required to be recorded. Broad reasons were contraindicated, intolerant or not applicable (i.e Angiotension Receptor blocker not prescribed as on ACEi or NYHA I symptoms therefore MRA indicated[2]). Between study visits any medication changes were noted. Specific Diabetes medication was recorded alongside the type of therapy (diet, oral hypoglycaemics, insulin, DDP inhibitor or GLP-1 inhibitor). Allergy status was also specifically recorded.

2.Procedure Data Collection

The CRT implantation was performed in a standard way as per UHCW standard operating procedure.

1. Type of CRT device (de novo vs upgrade)
2. Anatomical position of pocket
3. Model of pulse generator (model and company)
4. Vascular Access (veins, number of leads, number of punctures)
5. CS image recorded

6. RA lead (type, anatomical lead deployment position, lead fixation)
7. RA lead (type, anatomical lead deployment position, lead fixation)
8. LV Lead (type, lead fixation) circumferential position
    a. LV Lead position in CS (axial and circumferential position)
9. RA lead position (anatomical)
10. Immediate Complication (type and management)

For participants having a CRT-d, the reason for a defibrillator (primary or secondary prevention) was recorded.

Follow-up Study Visit Clinical Assessment

Overall the clinical data recorded during both follow study visit was the same as the baseline visit with the addition of examining CRT functional parameters and assessing for MACE . The clinical data capture form can be found in **Appendix K**.

Clinical Follow-up Data Collection

The clinical information collected was the same as the baseline study.

Medication History

The medication being taken at the time of the study visit is recorded in the same way as laid out in the baseline study visit. The emphasis during the follow-up study visits is to identify any changes to medication in the intervening periods.

Cardiovascular Outcome Data

Each follow-up visit was used to evaluate the patient for MACE. Specifically for HF hospitalisation episodes as defined in the projects MACE criteria.

APPENDIX K

Data Collection Forms

Initial Assessment

Participant Number: _____

Assessment Date: ____/____/_____

Consent Date: ____/____/_____

Demographics		
Date of Birth	__/___/___	
Gender	Male □	Female □
Background		
Ischaemic Cardiomyopathy:	Yes □	No □
Dilated Cardiomyopathy:	Yes □	No □
Heart Disease of other causes: _____		
Previous Myocardial Infarction	Yes □	No □
Previous CABG:	Yes □	No □
History of Hypertension	Yes □	No □
Diabetes	Yes □	No □
Clinical Information		

Current NYHA Class:	_____	
Does the participant have heart failure related symptoms?	Yes □	No □
If Yes then please apply:	Dysponea at rest □	
	Dysponea with minimal effort □	
	Dysponea withmoderate effort □	
	Nocturnal dysponea □	
	Ankle oedema □	
	Dyspepsia □	
	Syncope/ Pre-syncope □	
	Palpitations □	
	Fatigue □	
	Other, please specify:_____	
Drug History		
Allergies: _____		

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Implantation Data

Participant Number: _____

Implant Date: _____

Implant Data
Procedure Performed: _____
Pocket Position: _____
Device Implanted: _____
Puncture One Access:_____
Puncture Two Access:_____
Puncture Three Access:_____
Device Type:_____
Right Atrial Lead Type: _____
Lead Fixation: Active □ Passive □
Right Atrial Lead Position: _____
Right Ventricular Lead Type: _____
Right Ventricular Lead Position: _____
Lead Fixation: Active □ Passive □
Left Ventricular Lead Type: _____
Left Ventricular Lead Position in Coronary Sinus:

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Follow-up Assessment

Participant Number: _____

Assessment Date: ____/____/_____ Follow-up: 2 month □ 6 month □

Consent Date: ____/____/_____

Clinical Information		
Current NYHA Class:	_____	
Does the participant have heart failure related symptoms?	Yes □	No □
If Yes then please apply:	Dysponea at rest □	
	Dysponea with minimal effort □	
	Dysponea withmoderate effort □	
	Nocturnal dysponea □	
	Ankle oedema □	
	Dyspepsia □	
	Syncope/ Pre-syncope □	
	Palpitations □	
	Fatigue □	

Other, please specify:_____

CRT Follow-up

Complication Yes □ No □

If Yes , please specify:_____

Hospital admissions since CRT implantation? Yes □ No □

If Yes , please specify:_____

Percentage Biventricular Pacing: %

Battery Longevity:_____

Re-programmed? Yes □ No □

If Yes , please specify:_____

Repeat Echocardiogram organised? Yes □ No □

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University Hospitals Coventry and Warwickshire NHS Trust

Echocardiogram Data Collection Worksheet

Participant Number: _____

Assessment Interval: Initial / 2 months / 6 months

Assessment Date: _____

Parameter	Valve	Units
Height		cm
Weight		kg
Body Service Area		m2
Heart Rate		bpm
LV Dimensions		
LV internal end diastolic diameter (LVIDD)		mm
LV internal end systolic diameter (LVIDS)		cm
LV Volumes and Ejection Fraction		
End-diastolic volume (4-Chamber)		ml
End-systolic volume (4-Chamber)		ml
Ejection Fraction (LVEF) (4-Chamber)		%
End-diastolic volume (2-Chamber)		ml

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COVERT-HF: Body Composition Assessment

Participant Number: _____

Assessment Date: ____/____/_____

Assessment: Initial □ 2 months □ 6 months □

Body Composition	Value	Units
Age		
Height		cm
Weight		Kg
BMI		Kg/m2
Body Fat Percentage		%
Muscle Mass		Kg
Bone mass		Kg
Total Body Water Percentage		%
Visceral Fat Level		
Dietary Calorie Intake (DCI)		Kcal

Metabolic Age

Signature: _____

Date: ____/____/_____

APPENDIX L

Six Minute Walk Methods

Prior to the 6MWT, participants do not exercise or eat/drink for two hours prior to the test. A wheel chair was utilised to transport the participant between research equipment sites without requirement for performing exercise. A standard and clear explanation was provided to participants at each assessment according to the *American Thoracic Society* 2002 guidelines.[241] Opportunity was given to ask any questions. A 30 metre section of quiet flat corridor was marked out with two chairs at each end. Prior to starting the test heart rate and blood pressure were recorded. Participants were also required to rate symptoms of breathlessness and tiredness using the *Borg scale* (**Table L1**) Participants were required to walk between the chairs (**figure 5.6**). A continuous six-minutes was timed, and the participant was allowed to stop and rest as many times during the test as they required. The clock continued to run during any stops the participant made. During the test minimal communication was performed with the participant as per the *American Thoracic Society* 2002 guidelines.[241] Time checks were given every minute that elapsed The test was terminated on exactly 6 minutes, where the patient stopped and sat down. Immediate observations (as above) and symptoms were checked. Total distance walked is calculated based upon laps walked and final distance when stopped walking using a calorimeter. The participant was then asked to sit quietly for 15 minutes and was offered a glass of water.

Table L1. The Borg Symptoms Scale. Taken from American Thoracic Society; Am J Respir Crit Care Med 2002;.[241]

0 Nothing at all
0.5 Very, very slight (just noticeable)
1 Very slight
2 Slight (light)
3 Moderate
4 Somewhat severe
5 Severe (heavy)
6
7 Very severe
8
9
10 Very, very severe (maximal)

The change in 6MWT between pre-implant/baseline assessment and follow-up assessments is the measure of improvement and contributes to the functional composite response definition. A definition that has been used extensively to represent improvement following intervention on CHF patients and in particular CRT implantation is an improvement $\geq$10% 6MWT baseline distance.[1] This particular definition applied has been applied in a number of observational trials as being a criteria for a significant response/improvement in functional exercise capacity

.

APPENDIX M

LeftVentricle Dimensions and Function Quantification

Table M1. Left Ventricular Dimensions and Function Quantification. *LVEF catergorisation of severity reflects the British Society of Echoardiography measures.[200]. (Adapted[253])

	Women				Men			
	Reference Range	**Mild**	**Moderate**	**Severe**	**Reference Range**	**Mild**	**Moderate**	**Severe**
LV Dimension								
LVEDD (mm)	39-53	54-57	58-61	≥62	42-59	60-63	64-68	>69
LV Volume								
LVEDV (ml)	56-104	105-117	118-130	≥131	67-155	156-178	179-201	≥202
LVEDV/BSA (ml/m2)	35-75	76-86	87-96	≥97	35-75	76-86	87-96	≥97
LVESV (ml)	19-49	50-59	60-69	≥70	22-58	59-70	71-82	≥83
LVESV/BSA (ml/m2)	12-30	21-36	37-36	≥43	12-30	31-36	37-42	≥43
LV Function Two Dimensional Method								
LVEF (%)*	>55	45-54	36-44	≤35	>55	45-54	36-44	≤35

APPENDIX N

Participant Questionnaires

Warwick Medical School

University Hospitals Coventry and Warwickshire NHS Trust

Participant Questionnaire: '*The characterisation of circulating biomarkers before and after cardiac resynchronisation therapy in patients with heart failure and their role in predicting response'* (COVERT-HF)

Initial Assessment Questionnaire

Participant Number:

Please complete participant questionnaire:
Please circle the appropriate option where applicable:

Date of Recruitment:	Date of Assessment:
Age:	Gender: Male/ Female
Ethic Origin (Please circle appropriate) White British White Irish White other (please specify) Black African Black Caribbean Black British Black other (please specify) Asian other (please specify)	 Indian Pakistani Bangladeshi Chinese Asian British
Past Medical History:	Current Drug History:
1	1
2	2
3	3
4	4
5	5
6	6
7	7
8	8
9	9
10	10

APPENDIX O

Minnesota Living with Heart Failure ® Questionnaire Description

MLHFQ scoring has proven to have high reliability at baseline measurements.[328] Internal consistency, measured using Cronbach's alpha coefficient have also been shown to be consistently high.[328] The challenge with high internal consistency is a high score reflects a single construct, in the case of the MLHFQ the interrelated effects of CHF on an individual's QoL. However, the more inter-related the questionnaire the higher the internal consistency score will be, challenging the degree of validity. To overcome this challenge, comparing a questionnaire, to other constructs provides a bench mark to test the questionnaires validity. Gorkin *et al,*[328]demonstrated the *Functional Status score* (physical limitations) (r=0.75), *Dysponea scale* (r=0.52) and the *Clinician perceptions of patients health* (r=0.44) correlated highly with the MLHFQ score. Increasing NYHA classification has consistently demonstrated a strong and direct correlation with increasing MLHFQ scores.[28,258,328,329] The 6MWT distance reasonable correlates with the MLHFQ score (r=0.39).[330] Interestingly the MLHFQ score does correlates with LVEF poorly.[258,328]

The MLHFQ is a questionnaire designed to measure the impact of heart failure and its IMPACT on QoL.[258,259] Contained within the MLHFQ are key physical, emotional, social and mental dimensions, which are effected by heart failure and its treatment. There are 21 facets that are specifically explored; physical symptoms (shortness of breath, fatigue, peripheral oedema and difficulty sleeping), psychological impact (anxiety and depression)

and physical/social functioning (walking, climbing stairs, household work, need to rest, working to earn a living, going place places away from home, doing things with friends or family, recreational activities, sexual activities, eating and mental and emotional functions of concentrations, memory, loss of self-control and being a burden to others).[258,259] Each facet/question has the same six –point scale, between 0 and 5, where 0 means not at all and 5 means very much. The MLHFQ provides a total score (range 0–105, from best to worst QoL).[258,259] Furthermore, two QoL dimensions are measured; physical (8 items, range 0–40) and emotional (5 items, range 0–25).[258,259] The eight remaining facets are only considered for the calculation of the total score.[258,259] A 'clinically meaningful' difference is the change in score between tests that indicates a large enough change for physicians and patients to consider using a new treatment, if risks and costs were acceptable.[257] A score change of five-points is the minimum needed to define a significant change in QoL.[331] MLHFQ has been extensively used in pharmcology[332,333] and CRT[23,28,250] intervention randomised control trials and proven to be robust measure of total QoL in CHF intervention trials.

APPENDIX P

Minnesota Living with Heart Failure Qestionnaire®

The following questions ask how much your heart condition has affected your life during the past month (4 weeks). After each question, circle the 0, 1, 2, 3, 4 or 5 to show how much your life was affected. f a question does not apply to you, circle the 0 after that question.

Has your heart condition prevented you from living as you wanted during the last month (4 weeks) by

	No	Very Little				Very Much
1. Causing swelling in your ankles or legs?	0	1	2	3	4	5
2. Making you sit or lie down to rest during the day?	0	1	2	3	4	5
3. Making walking about or climbing stairs difficult?	0	1	2	3	4	5
4. Making working around the house or garden difficult?	0	1	2	3	4	5
5. Making going places away from home difficult?	0	1	2	3	4	5
6. Making sleeping well at night difficult?	0	1	2	3	4	5
7. Making doing things with your friends or family difficult?	0	1	2	3	4	5
8. Making working to earn a living difficult?	0	1	2	3	4	5
9. Making your recreational pastimes, sports or hobbies difficult?	0	1	2	3	4	5
10. Making sexual activities difficult?	0	1	2	3	4	5

Has your heart condition prevented you from living as you wanted during the last month (4 weeks) by

	No	Very Little				Very Much
11. Making you eat less of the foods you like?	0	1	2	3	4	5
12. Making you short of breath?	0	1	2	3	4	5
13. Making you tired, fatigued, or low on energy?	0	1	2	3	4	5
14. Making you stay in a hospital?	0	1	2	3	4	5
15. Costing you money for medical care?	0	1	2	3	4	5
16. Giving you side effects from treatments?	0	1	2	3	4	5
17. Making you feel you are a burden to your family or friends?	0	1	2	3	4	5
18. Making you feel a loss of self-control in your life?	0	1	2	3	4	5
19. Making you worry?	0	1	2	3	4	5
20. Making it difficult for you to concentrate or remember things?	0	1	2	3	4	5
21. Making you feel depressed?	0	1	2	3	4	5

Thank you taking the time to complete the questionnaire. Any questions please do not hesitate to contact Dr Christopher McAloon on 02476 965 813.

APPENDIX Q

Air-Displacement Plethysmography (BOD-POD®)

BOD POD® is comparable with other more traditional body composition methods include hydrostatic weighing and dual energy X-ray absorptiometry.[261] Components of body composition measured are FM, FFM and body fat percentage. BOD POD® has been demonstrated to be a reliable measure of body composition. When measuring the same 50 litre cylinder 20 times consecutively, the reliability is excellent; demonstrated by a coefficient variant of 0.025%.[261] Reported reliability of serial measurements on the same in humans demonstrated a coefficient variant ranging from 1.7% to 4.5% for body fat.[261] Measurements performed between days ranged from 2.0-2.3%.[261]Comparing repeated measurements of body fat percentage on different days between BOD POD® and hydrostatic weighting showed there was no significant difference.[261] The BOD POD® has also been demonstrated to have higher precision in measurements of body volume than hydrostatic weighting techniques.[261] Variations when using BOD POD® have been observed between genders, body habitus and age.[261] However, no data to date has been undertaken to validate BOD POD® use in HF patients.[261] Importantly BOD POD® has no contraindications to use in patients with a CRT *in situ*. Overall BOD POD® is a reliable and well validated measure of body composition. [261] BOD POD also measures body water as fat mass, which in HF patients is an important confounder in the assessment of body composition. However, patients in the study were stable and decompenstated patients were not enrolled in the study.

APPENDIX R:

Selection of Published Abstracts

ACC.15
TCT@ACC-i2 | innovation in intervention

A461
JACC March 17, 2015
Volume 65, Issue 10S

Arrhythmias and Clinical EP

COMPARISON OF UPGRADED VERSUS DE-NOVO CARDIAC RESYNCHRONISATION THERAPY (CRT) DEVICES ON CARDIOVASCULAR OUTCOMES AND RESPONSE: A 5 YEAR REGISTRY

Poster Contributions
Poster Hall B1
Monday, March 16, 2015, 9:45 a.m.-10:30 a.m.

Session Title: Outcomes and Cardiac Device Therapy
Abstract Category: 6. Arrhythmias and Clinical EP: Devices
Presentation Number: 1253-248

Authors: *Christopher James McAloon, Domonic P. Heining, Jethro J. Barker, Benjamin Anderson, Gavin Atherton, Faizel Osman, University Hospital Coventry and Warwickshire NHS Trust, Coventry, United Kingdom, Warwick Medical School, University of Warwick, Coventry, Coventry, United Kingdom*

Background: CRT is an effective therapy in HF. Pacemakers and defibrillators are implanted for arrhythmogenic indications. Pacing dependency can induce HF and is an established CRT upgrade criterion [LVEF<35% and ventricular pacing >40%]. We aimed to compare cardiovascular outcomes between upgraded and de-novo CRT's.

Methods: A retrospective study of all consecutive CRT implants over five years (Jan 2009 - Dec 2013) in a UK tertiary centre. Data was collected on baseline demographics, co-morbidities and indications (NYHA class, ECG, echocardiogram). Comparisons were made for these outcomes: acute response [2mths], all-cause mortality and first hospitalisation. CRT response was defined as >1 NYHA class increase.

Results: 373 CRT implants were performed (79 upgrades). Upgrade and de-novo patients were matched for all comorbidities. Table 1 demonstrates baseline demographics, indications and CRT response. A Kaplan-Meier analysis was performed on all-cause mortality and time to first hospitalisation, with three year rates demonstrated in the table. A significantly worse long-term response (p0.002) and all-cause mortality (p0.043) was demonstrated for upgrade patients.

Conclusion: Upgraded CRT patients have a worse long-term response and higher all-cause mortality. The potential worse outcome for upgrade patients should be considered before implant.

HF = Heart Failure, NYHA = New York Heart Association, LVEF = left ventricular ejection fraction, ECG = Electrocardiogram

Table 1

	Total Cohort N=373	Upgrades N= 79	De Novo Implants N=294	P value
Age (mean+/-SD)	72.0+/-10.4	74.0 +/- 10.6	71.44 +/- 10.4	0.055
Gender (male n,%)	287 (76.9%)	69 (87.3%)	218 (74.1%)	0.013
Device (CRT-D n,%)	182 (48.8%)	39 (49.4%)	143(48.6%)	0.909
NYHA Class 3/4 (n,%)*	324 (91.5%)	74(98.7%)	250 (90.0%)	0.028
LVEF <35% (n,%)*	364 (98.4%)	76(97.4%)	288(98.6%)	0.458
QRS >150msec (n,%)*	272 (77.1%)	55 (78.6%)	217 (76.7%)	0.634
Acute Response (n,%)	132 (61.4%)	23 (59.0%)	109 (61.9%)	0.731
Long-term Response (n,%)	101 (47.0%)	11 (25.6%)	90(52.3%)	0.002
All-Cause Mortality Rate (36 months) (%+/-SE)	21.1%+/-2.6%	25.6%+/-5.8%	19.2%+/-2.9%	(Log-Rank) 0.043
All-Cause First Hospitalisation (36 months) (%+/-SE)	42.9%+/-3.3%	46.7%+/-3.7 %	41.9%+/-4.2 %	(Log-rank)0.096
*Percentage represents all recorded data				

Abstract 53 Table 1 Device implantation according to device

Device	PPM	ICD	CRT-P	CRT-D	Device upgrade	Total
Number implanted	705	339	129	246	66	1419
Pneumothorax (%)	13 (1.8)	4 (1.25)	2 (1.6)	0	0	19 (1.3)
Lead displacement						
Patients (%)	31(4.4)	20(5.9) ⌘	2 (1.6)	13 (5.3)	3 (4.5)	68 (4.8)
RA	13* (1.8)	8 (2.3)	0	4 (1.6)	1 (1.5)	26 (1.8)
RV	22" (3.1)	13 (3.8)	0	2 (0.8)	0	37 (2.6)
LV	0		2 (1.6)	7 (2.8)	2 (3)	11 (0.8)
Wound problem						
Haematoma (%)	3 (0.4)	1 (0.3)	0	0	0	4 (0.3)
Infection (%)	2 (0.3)	4$ (1.2)	0	1 (0.4)	1 (1.5)	8 (0.6)
Painful pocket (%)	1 (0.1)	2 (0.6)	0	1 (0.4)	0	4 (0.3)
Effusion						
Conservatively managed	4 (0.6)	0	0	0	1 (1.5)	5(0.35)
Tamponade (requiring drain)	1 (0.1)	0	0	0	0	1(0.07)

*One patient had RA lead reposition followed by RA lead replacement
"One patient had RV lead reposition followed by RV lead replacement
⌘One patient had both RA and RV lead displacement, both repositioned at the same procedure
$includes 2 post lead revision and 1 post new system post extraction

bradyarrhythmias, loss of pacing function when pacing-dependent or inappropriate defibrillator shocks. The were no device-related deaths or complications requiring ITU level care.

Conclusion Same day discharge appears safe in an unselected population of patients undergoing elective primary implantation of a CRM device at a high-volume cardiothoracic unit. Procedural difficulties, symptoms or signs suggestive of a potential complication should prompt further evaluation, and all patients should undergo device interrogation and chest radiography prior to discharge.

54 MEASURES OF ENDOTHELIAL DYSFUNCTION PREDICT RESPONSE TO CARDIAC RESYNCHRONISATION THERAPY

[1]David Warriner*, [2]Ryan Crapper, [1]Patricia Lawford, [3]Paul Sheridan. [1]*Sheffield University*; [2]*Sheffield Teaching Hospitals*; [3]*Chesterfield Royal Infirmary*; **Presenting Author*

10.1136/heartjnl-2016-309890.54

Background Cardiac resynchronisation therapy (CRT) improves morbidity and mortality in heart failure (HF). Impaired endothelial function, as measured by flow mediated dilation (FMD) is associated with increased morbidity and mortality in heart failure (HF) and may help to differentiate responders from non-responders.

Methods FMD was measured at baseline and 12 months following CRT. The patient group were 94% male, mean age 69 ± 8 years, New York Heart Association (NYHA) functional class II-IV, QRSd 173 ± 21 ms and had a left ventricular ejection fraction (LVEF) 26 ± 8%.

Results 70% of patients were found to have responded at 12 months. Responders had significant improvements in VO_2 (12.6 ± 1.7 to 14.7 ± 1.5 ml/kg/min, $p < 0.05$), quality of life score (43 ± 23 to 24 ± 22, $p < 0.01$), left ventricular end diastolic volume (210 ± 125 ml to 173 ± 125 ml, $p < 0.01$), NT-proBNP (2422 ± 829 ml to 1732 ± 976 ml, $p < 0.01$ and 6 min walk distance (379 ± 117 m at baseline to 418 ± 105 m, $p < 0.05$). Baseline FMD in responders was 2.9 ± 1.9% and 7.4 ± 3.73% in non-responders ($p < 0.05$).

Conclusions This confirms that FMD identifies response to CRT, due to endothelium dependent mechanisms alone.

55 EVALUATION OF POTENTIAL CLINICAL RESPONSE AND CARDIOVASCULAR OUTCOMES PREDICTORS IN A TERTIARY CARDIAC RESYNCHRONISATION THERAPY IMPLANTATION CENTRE

Christopher McAloon*, Dominic Heining, Gavin Atherton, Benjamin Anderson, Harpal Randeva, Paul O'Hare, Faizel Osman. *University Hospital Coventry and Warwickshire*; **Presenting Author*

10.1136/heartjnl-2016-309890.55

Background Cardiac Resynchronisation Therapy (CRT) is an effective treatment for dys-synchronous chronic heart failure (CHF), however there is a significant non-response rate. Clinic predictors of response and cardiovascular outcome are often inconsistently reported. The aim of the study was to examine previously reported clinical predictors of response and cardiovascular outcomes in a heterogeneous CHF patients undergoing CRT implantation at a UK tertiary centre.

Methods A retrospective single-centre cohort study of all consecutive CRT implantations (147 (49.0%) CRT-p; 153 (51.0%) CRT-d) performed over 5 years (Jan 2009–Dec 2013). Implants had to meet eligibility criteria; successful implant, follow-up case records availability and clinical response determination. Clinical response was defined by three independent reviewers as a New York Heart Association classification symptom reduction > 1 class or class I maintenance from pre-

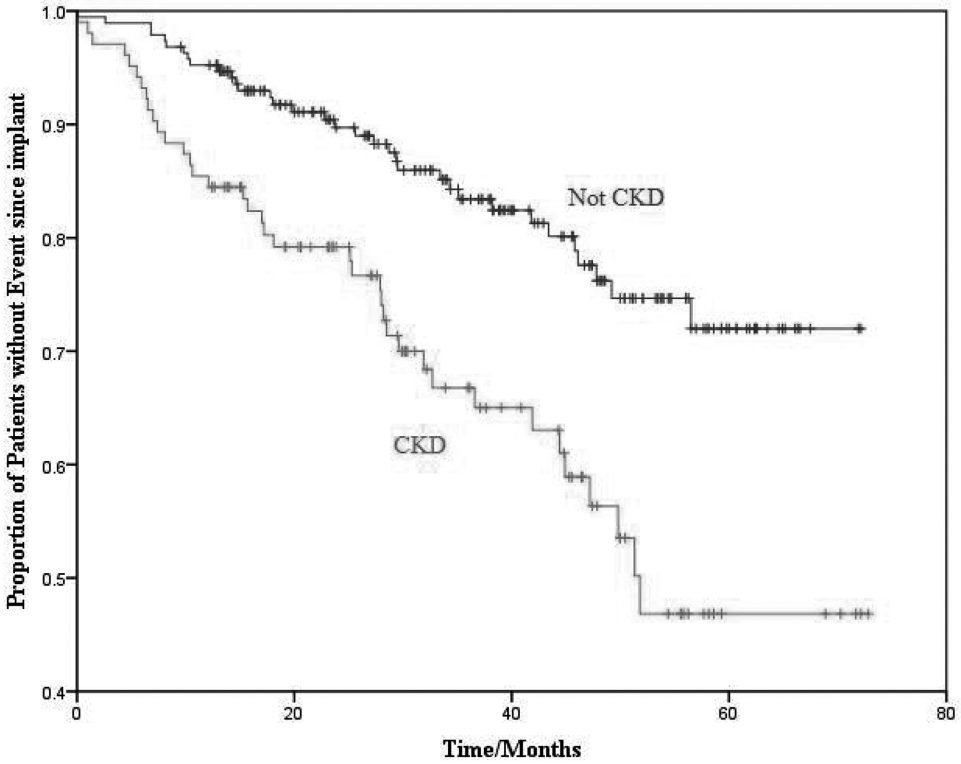
implant to the most recent cardiology/heart failure consultation. Major Adverse Cardiovascular Events (MACE), defined as all-cause mortality or first heart failure hospital admission, was recorded independently of clinical response. Pre-identified potential clinical predictors (Table 1) were analysed to determine ability to predict response and MACE.

Results A cohort of 300 (mean age 71.5 years ± 10.1; 227 (75.7%) males) had clinical response definable (158 (52.7%) responders; 142 (47.3%) non-responders) at a median of 12.0 (± (IQR) 4.38–25.5) months. Baseline cohort characteristics were: 171 (59.0%) ischaemic aetiology; 72 (28.0%) AF; 75 (25.9%) Diabetes; 103 (25.3%) Chronic Kidney Disease (CKD); Electrocardiogram: QRS 154mesc (± 144–172); 186 (71.8%) LBBB; Echocardiogram 24.1% (± (SD)8.3) LVEF. Multivariate logistic regression (Table 1) of pre-defined parameters of overall clinical response demonstrated increasing age at implant predicted a poorer response (OR 0.96, *p*0.002, CI (95%) 0.94–0.99). CKD status trended towards predicting long-term (>12 weeks) clinical response (OR 0.58, *p*0.06, CI (95%) 0.33–1.01). Figure 1 shows the survival curve demonstrating significantly higher all-cause mortality rate for those with CKD at implant ($p < 0.001$). Multivariate Cox regression demonstrated that CKD status predicted increased MACE (HR 2.10, p0.001, CI (95%) 1.23–3.19) and all-cause mortality (HR 2.06, $p < 0.007$, CI (95%) 1.22–3.46) following CRT implantation.

Abstract 55 Table 1 Univariate and multivariate logistic regression of potential predictors of overall clinical response

	Univariate Regression			Multivariate Regression		
Predictor	**Odds Ratio**	**p Valve**	**Confidence Interval (95%)**	**Odds Ratio**	**p Valve**	**Confidence Interval (95%)**
Age at implant	0.97	0.00	0.94–0.99	0.96	0.002	0.94–0.99
Gender	0.96	0.88	0.57–1.63			
Device	1.41	0.14	0.90–2.22			
Upgrade Status	0.54	0.03	0.31–0.95	0.57	0.05	0.32–1.01
QRS Duration	0.87	1.00	0.99–1.01			
LBBB	1.42	0.20	0.84–2.42			
LVEF	0.98	0.21	0.95–1.01			
Aetiology	1.18	0.50	0.74–1.87			
Diabetes Mellitus	0.84	0.53	0.50–1.43			
CKD	0.60	0.04	0.37–0.97			
Days from Implant to Response Assessment	1.00	0.13	0.99–1.00	0.99	0.03	0.99–1.0

Conclusion Increasing age at implant predicts poorer overall clinical response. CKD status predicts increased MACE and all-cause mortality events following CRT.



Month	0	20	40	60
CKD	103	73	34	5
Not CKD	189	141	76	18

Abstract 55 Figure 1 Survival curve for CKD status at CRT implantation and all-cause mortality ($p < 0.001$)

73

TROUBLESHOOTING LV LEAD IMPLANTATION - NOVEL "UNIRAIL TECHNIQUE"

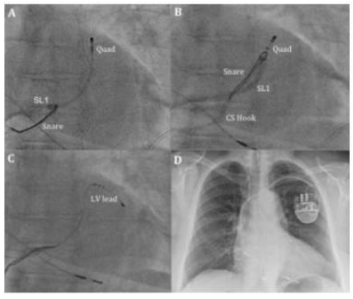
S. Honarbakhsh, P.D. Lambiase, M.D. Lowe, R.J. Hunter, R.J. Schilling, and M. Finlay

Barts Heart Centre, Barts Health NHS trust, London

Introduction: Lack of guide catheter support and difficulty accessing the coronary sinus (CS) are frequently cited as reasons for difficulty in placing CS pacing leads. We present a new "unirail" technique whereby a femorally placed catheter provides the support for guide-catheter placement at the os of a target vessel.

Method and results: A 69 year old female was referred for device upgrade. She was pacing dependent, had moderate left ventricular (LV) impairment, and had NYHA class III symptoms. Despite utilizing a range of established techniques (including balloon anchoring), it was impossible to pass a CS lead into any target vessel due to lack of guide-catheter support. She was rescheduled for a further procedure where a combination of subclavian and femoral access was used. A CS hook guide was advanced from the subclavian vein, whilst a quadripolar electro-physiology catheter (quad) was simultaneously advanced through a femoral SL1 sheath, which remained within the low right atrium. A gooseneck snare passed from the CS hook lassoed the quad and the quad was then advanced into the CS (figure 1A). The CS hook sheath was advanced, tightening the snare around the quad at the CS os position. The SL1 sheath was then advanced over the quad, railing the lassoed snare and CS hook up the path of the CS to the os of the target vein (figure 1B). The quad was then withdrawn, releasing the snare. A Starfix LV lead was then easily positioned into the target vessel without complications (figure 1C and D). At four month follow-up pacing parameters were stable. Patient was NYHA class I and LV function had improved to mildly impaired.

Discussion: This case illustrates a novel technique of accessing complex coronary anatomy. Minor variations of this method would allow the guide-cath to be supported from the femoral vessel whilst placing a lead if required.



74

SUBCLINICAL ATHEROSCELEROSIS AND COGNITIVE IMPAIRMENT

Sevara Tairova, Gulnara Rakhimbaeva, and Nigora Akramova

Tashkent Medical Academy, Tashkent

Purpose: To determine associations between subclinical atherosclerosis, cognitive screening and white matter hyperintensities on MRI.

Method and materials: The study consisted of 124patients from the department of neurology of Tashkent Medical Academy(mean age 44 + 10 years, 56% female and 44% male) without cardiovascular disease who underwent carotid and brain MRI at 3 Tesla. Semi-automated tech-niques were used to define wall contours of the internal and common carotid arteries (ICA and CCA) and white matter hyperintensity volume (WMH). Subjects also underwent Montreal Cognitive Assessment (MoCA) testing and muHidetector CT for measurement of coronary artery calcium (CAC) using the Agatston method. A MoCA score less than 26 was used to indi-cate the presence of at least mild cognitive impairment.

Results: ICA and CCA wall areas correlated with WMH and MoCA score (all p , .001) in unadjusted models. After adjusting for traditional risk factors, ICA wall area remained asso-ciated with MoCA(¼0.02, p , .05) and CCA wall area remained associated with WMH (0.002, p ¼ 0.04). Increasing ICA wall area predicted MoCA score ,26 (OR 1.12 per SO change, 95% Cl 0.99-1.26, p ¼ 0.04) after multivariable adjustment, but increasing CCA wall area did not predict MoCA score ,26 (p ¼ 0.5). After adjusting for traditional risk factors, CAC was associated with WMH (¼0.013, p ¼ O.OOOB). Increasing CAC score predicted large WMH (OR 1.19 per SO change, 95% Cl 1.03-1.38, p ¼ 0.02).

Conclusion: Subclinical coronary and carotid atherosclerosis are predictors of poorer cognitive function as measured by MoCA score and white matter hyperintensity volume on MRI.

75

EFFECT OF LOZARTANE ON DEVELOPMENT OF THE ELECTRICAL INSTABILITY OF THE MYOCARDIUM

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Aim: to study the effect of Lozartane on the morphologically functional characteristics of the patients with various dysfunctions of the heart rhythm.

Materials and methods: The study included 62 patients aged 50 to 70. (57.4 + 1.82 years) with following diag-noses: ischemic heart disease (stable pressure angina, types I-III) (80.7% of patients), heart failure of types I-IIA (90.3% of patients), hypertonic heart disease of types I-II (87.1% of patients), myocardial fibrosis (12.9% of patients). Changes to the heart rhythm were identified in all of the patients: 36% of patients suffered from ventricu-lar beats, 29.6% with supraventricular beats, 44.8% with paroxysmal and persistent forms of atrial fibrillation and 27.4% with constant form of atrial fibrillation. All patient received the basic therapy, which included Lozartane. The research was conducted twice: with admission of the patient to the hospital and 4 weeks after the Lozartane-consisting treatment. The following methods were used: clinical observation; ECG-control with measurement of the QT interval; Holter monitoring; 24-hour monitoring of AD and echocardigraphy.

Results of the study

Factor	Initial value		After the treatment	P
Mean HR (bpm)	78,06	+ 2,57	71,71 + 3,84	0,00001
Maximum HR (bpm)	125	+ 4,44	102,43 + 8,44	0,00001
Minimum HR (bpm)	51,6	+ 2,86	46,71 + 2,78	0,00001
Ventricular beats (absolute quantity)	443,4	+ 205,84	169,35 + 103,94	0,00001
Supraventricular beats (absolute quantity)	417,97	+ 133,26	216,78 + 122,9	0,00001
FP runs (absolute quantity)	32,35	+ 26,3	16,38 + 14,93	0,0006
SDNN (ms)	99,26	+ 9,25	127,37 + 12,86	0,00001
SDANN (ms)	123,32	+ 11,77	127,95 + 11,1	0,0555
SDNNi (ms)	59,06	+ 6,12	61,6 + 7,89	0,2273
RMSSD (ms)	48,82	+ 8,83	58,38 + 14,95	0,0003
pNN50 (%)	15,79	+ 3,89	18,05 + 5,06	0,0189
LF (ms^2)	2124,74	+ 708,82	1322,0 + 517,06	0,00001
HF (ms^2)	1641,32	+ 620,52	1281,5 + 614,77	0,0064
QTc (ms)	418,4	+ 14,95	414,08 + 40,28	0,00001
DQT (ms)	17,26	+ 1,68	16,48 + 2,15	0,0518

Conclusions: Significant difference in the decrease of the corrected QT interval and dispersion of QT interval, along with positive effect of Lozartane on the values of variable heart rhythm, allow to correlate the antiarrhyth-mic effect of the medication with its positive effects on the vegetative regulation of the heat tone, as well as with the independent antiarrhythmic activity of the Lozartane metabolite, EXP3174.

c device.

76

THE INTERPLAY BETWEEN BODY COMPOSITION AND LEFT VENTRICULAR REMODELLING IN CARDIAC RESYNCHRONISATION THERAPY

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Background: Body composition (BC) alters in heart failure (HF) patients. Cardiac resynchronisation therapy (CRT) improves left ventricular (LV) geometry, but impact on BC is unknown.

Purpose: To examine BC changes in HF patients undergoing CRT and interplay with clinical, neurohormonal and echocardiographic parameters.

Method: Prospective cohort study of HF patients undergoing CRT [meeting ESC guidelines] between Sept14-Dec15. Each participant underwent 3 assessments [pre-CRT, 6 weeks, 6 months] for BC parameters [air-displace-ment plethysmography], New York Heart Association (NYHA), echocardiographic parameters [LV End Systolic/ Diastolic volume (LVESV/LVEDV), LVESV index (LVESVi), LV ejection fraction (LVEF)], electrocardiog-raphy, Minnesota Living with HF Questionnaire (QOL), and N-terminal pro-brain natriuretic peptide (NT-pro-BNP). Repeated Measures Analysis of Variance was performed to assess parameter's change over time and corre-lations of parameters explored.

Results: 25 participants recruited; 73.4 + 10.0 years (mean + standard deviation), 23 males, 18 CRTd, 16 Ischaemic, 6 Diabetes, 17 LBBB and 10 Atrial Fibrillation. During follow-up there was 1 HF mortality, 2 unable to attend (1 HF hospital admission), 1 LV lead displacement and 1 unable to undertake BC assessment after CRT. Table 1 demonstrates the trend in the change in parameters over 6 months follow-up. Alterations between other BC and LV parameters in first 6 weeks also strongly correlated; Fat Mass (FM) and LVESVi (r ¼ -0.76,p , 0.001), Lean Mass vs LVESVi (r ¼ 0.63, p ¼ 0.008), FM vs LVEDV (r ¼ -0.66, p ¼ 0.005) and FM vs LVEF (r ¼ 0.49, p ¼ 0.06).

Conclusion: This is the first study to demonstrate interplay between BC and LV geometry alterations following CRT. A trend in overall FM reduction is also suggested. BC in CRT requires further study.

Table 1.

	Baseline (n=25)	6 weeks (n=23)	6 months (n=20)	*p-value*
NYHA+	3 (2-3)	2 (2-3)	2 (2-3)	*<0.01*
QOL Score#	42.2±20.6	31.2±21.9	31.5±24.6	*0.06*
QRS (msec)#	163.9±20.6	147.5±25.6	158.23.6	*<0.01*
NT-pro-BNP (pmol/L)+	267.0 (118.5-353.5)	236.5 (84.8-517.5)	272.5 (91.0-598.8)	*0.87*
BMI (kg/m2)#	29.7±5.5	28.6±4.3	28.5±4.4	*0.24*
Fat Mass (kg)#	34.3±12.8	30.9±9.8	31.4±7.9	*0.14*
Lean Mass (kg)#	52.1±9.7	52.1±9.8	50.9±10.0	*0.31*
LVEF (%)#	25.9±7.4	29.8±8.5	30.8±8.4	*0.24*
LVESVi (ml/m2)#	65.25±24.2	56.5±20.8	57.6±32.9	*0.05*

means±SD, + median (inter-quartile range)

APPENDIX S

Published Articles

Safety and Cost-Effectiveness of Same-Day Cardiac Resynchronization Therapy and Implantable Cardioverter Defibrillator Implantation

Gavin Atherton, BSc (Hons), MBChB[a], Christopher James McAloon, MBChB, MRCP, PGCME[a,b], Bhaveek Chohan, BSc (Hons)[a], Dominic Heining, MSc, MBChB[a], Benjamin Anderson, MBChB, MRCP[a], Jethro Barker, BSc (Hons)[a], Harpal Randeva, MBChB, PhD[a], and Faizel Osman, MBChB, MD[a,b,*]

Cardiac resynchronization therapy (CRT) and implantable cardioverter defibrillator (ICD) implantation improve morbidity and mortality in selected patients. Many centers still admit patients overnight. We evaluated the safety, feasibility, and cost savings of same-day CRT/ICD device implantation by performing a retrospective study of all consecutive elective CRT/ICD implants at a tertiary center from January 2009 to April 2013. All emergency and/or inpatient cases were excluded. Data were collected on baseline demographics, implantation indication, procedure details, complications (categorized as immediate [≤24 hours], short term [24 hours to 6 weeks], medium term [6 weeks to 4 months], and long term [>4 months]), and mortality (30 day and 1 year). Comparisons were made between those having planned same-day versus overnight stay procedures. A cost analysis was performed to evaluate cost savings of the same-day policy. A total of 491 devices were implanted during this period: 267 were elective (54 planned overnight, 213 planned same-day) of which 229 were CRT pacemakers or CRT defibrillators and 38 ICDs. There were 26 total overall complications (9.7%) with no significant differences between planned same-day versus planned overnight stay cohorts (9.4% vs 11.1%, p = 0.8) and specifically no differences in immediate, short-, medium-, and long-term complications at follow-up. The 30-day and 1-year mortality rates did not differ between the two groups. An overnight stay at our hospital costs $450 (£300); our cost saving during this period was $91,800 (£61,200). Same-day CRT/ICD implantation is safe, feasible, and associated with significant cost savings. It provides significant advantages for patients and health care providers, especially given the current financial climate. **(Am J Cardiol 2016;■:■−■)**

Cardiac resynchronization therapy (CRT) and the implantable cardioverter defibrillator (ICD) are known to improve morbidity and mortality in selected patients.[1−3] CRT has been shown to reduce mortality and hospitalization in selected patients with heart failure on optimal medical therapy.[2,4,5] ICDs implanted for primary and secondary prevention have become the cornerstone in the prevention of sudden cardiac death in selected patients.[3,4] Recent changes to international guidelines reflect the success of these devices and are now offered to a wider range of patients[6,7] with implantation rates continuing to rise year on year.[8] The cost-effectiveness of such procedures has been an important issue of discussion.[9,10] Many centers in Europe and North America keep patients overnight after implantation, driven mainly by the assumed risk of device-related complications. With increasing health care costs and the current worldwide financial climate, the cost−benefit ratio of such a strategy is now being reconsidered. We have previously reported that same-day bradycardia pacemaker implantation is safe and cost-effective with significant cost savings for health care providers.[11] The aim of the present study was to evaluate the safety and feasibility of this same-day policy by comparing outcomes with those routinely kept overnight; we also performed a cost-saving analysis for the period of study.

Methods

A retrospective study of all consecutive elective CRT pacemaker (CRTp), CRT defibrillator (CRTd), or ICD (single/dual chamber) implants performed at University Hospital Coventry, UK, from January 2009 to April 2013. This included all de novo and upgrade cases but excluded all having a pulse generator change only (as these patients are not usually admitted overnight). Elective implants were those seen in the outpatient clinic and subsequently admitted with a planned date. We excluded all who had a device implanted during an acute emergency hospital admission. Electronic patient records and case notes were reviewed for

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See page 5 for disclosure information.

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all cases. Data were collected on patient demographics, implant indication, procedure details, and outcomes. Patients were divided into 2 cohorts: planned same-day implants (following commencement of our same-day policy in 2010) and those planned to be kept overnight (patients implanted before same-day policy). Patients were seen routinely at 6 weeks and 4 months after the implant. Approval for the study was provided by our Local Audit and Research Department. The study applied the principles of the declaration of Helsinki. Complications were categorized overall and by time of occurrence: immediate (≤24 hours), short term (24 hours to 6 weeks), medium term (6 weeks to 4 months), and long term (>4 months, median 30 months, interquartile range 20 to 42 months). Mortality was examined at 30 days and 1 year after the procedure and classified as due directly to the device implant, cardiac cause (heart failure, myocardial infarction, arrhythmia, sudden cardiac death), and non-cardiac causes. Types of complication recorded were cardiac arrest, cardiac chamber perforation, tricuspid valve injury, pneumothorax or hemothorax, stroke, myocardial infarction, pericardial effusion or tamponade, major bleeding (defined as decrease in hemoglobin ≥20 g/l and/or blood loss requiring blood transfusion), wound hematoma (with or without reintervention), coronary sinus (CS) dissection or perforation (for CRT implants), procedure- or device-related infections, lead displacement, phrenic nerve stimulation postoperatively for CRT cases, and device erosion. Failed procedures due to patient factors (such as poor CS anatomy, poor patient tolerance) were also recorded. Both upgrade and de novo device implants were evaluated because of the defined increase in complication rates reported in upgraded devices.[12]

All implants during the period of study were performed by a consultant cardiologist with specialist interest in EP/Devices alone or scrubbed with a Devices Fellow. Patients on oral anticoagulation were asked to discontinue it 3 days before operation and resume it the same day after operation. Conscious sedation (mostly midazolam 0.1 mg/kg intravenously) and local anesthesia (1% lidocaine) was used in all patients with a left deltopectoral groove incision in most. Venous access was mainly cephalic and axillary veins with subclavian vein used only if the first 2 routes failed. The right ventricular lead for most patients was implanted at the right ventricular apex and right atrial lead at the right atrial appendage. Patients in permanent atrial fibrillation did not have an atrial lead implanted. For left ventricular (LV) lead implantation, the CS was cannulated, and angiography was performed in all with the most lateral and basal/midcavity position favored; redundant leads were capped and left in situ. The pulse generator was placed into a prepectoral pocket. Defibrillation safety margin testing was not performed. All were given preprocedural intravenous antibiotics using 1-g flucloxacillin and 1.5-mg/kg gentamicin (maximum dose 150 mg) and 3 days after procedural oral antibiotics (flucloxacillin 500 mg 4 times a day). Patients with penicillin allergy were given intravenous teicoplanin 600 mg preoperatively, and oral doxycycline 200 mg/day for 3 days postoperatively. All had postimplant chest x-ray to check lead position and exclude pneumothorax. Patients were taken back to the ward and kept overnight for observation before our same-day protocol. Following the same-day protocol, patients were taken to our cardiology day-case unit and observed for 3 to 4 hours before being discharged if all checks were satisfactory. If for any reason there were concerns about same-day discharge, patients were admitted overnight and discharged the following day. Those discharged were instructed not to undertake strenuous physical activity for 1 week after and advised to keep the wound dry for 3 days postoperatively. In case of problems, patients were instructed to contact our day-case unit or cardiology ward immediately. Those living farther away were listed earlier than those living closer to allow timely discharge. Age and geography were not specifically used to exclude patients. The costing for an overnight stay in a cardiology bed was obtained from our finance department. We calculated actual cost savings that occurred during the period of study for those patients who were discharged home the same day only.

Statistical analysis was performed using Statistical Package for Social Sciences [SPSS], version 22.0 (IBM, Chicago, Illinois). Categorical variables were reported as numbers and percentages. Comparison analysis for categorical data was performed using the chi-square test. Where smaller than expected values were derived, Fisher's exact test was performed. Continuous data underwent histogram plots and Kolmogorov−Smirnov tests of normality. Normally distributed data were reported as mean ± SD. Non-normally distributed data were reported as median ± interquartile range. Comparison analysis for independent normally distributed continuous data was performed using the unpaired t test. Independent continuous data that were not normally distributed underwent comparison analysis using the Mann−Whitney U test. A value of $p < 0.05$ was used for statistical significance.

Results

There were 491 patients who underwent a complex device procedure during the period of study (Figure 1). Of these, 224 (53 CRTd, 40 CRTp, 131 ICD) were done during an acute hospital admission and were excluded from analysis leaving 267 elective procedures (114 CRTd, 115 CRTp, 38 ICD). Of these, 54 (20%) were planned overnight and 213 (80%) planned same-day cases. Baseline characteristics of the 2 cohorts are summarized in Table 1 with no significant differences noted. In the same-day cohort, there were 9 (4.2%) unplanned overnight hospital admissions: 8 CRT (CRTp = 5, CRTd = 1, CRTp upgrade = 2) and 1 single chamber ICD. Three were kept in because of a complication (pneumothorax, wound hematoma); the remaining were kept in at the operators discretion (late finish = 2, multiple co-morbidity/social reasons = 2, generally unwell after procedure with no specific complication = 2). These patients were analyzed as part of the same-day cohort when comparing groups; however, they were excluded from analysis of actual cost savings as they were admitted overnight.

Successful device implantation (satisfactory lead and device implantation) occurred in 260 (97.3%) of the entire cohort. The 7 failed implants were all CRT (CRTp = 2, CRTp upgrade = 3, CRTd = 1, CRTd upgrade = 1) with reason for failure in all being inability to securely implant an

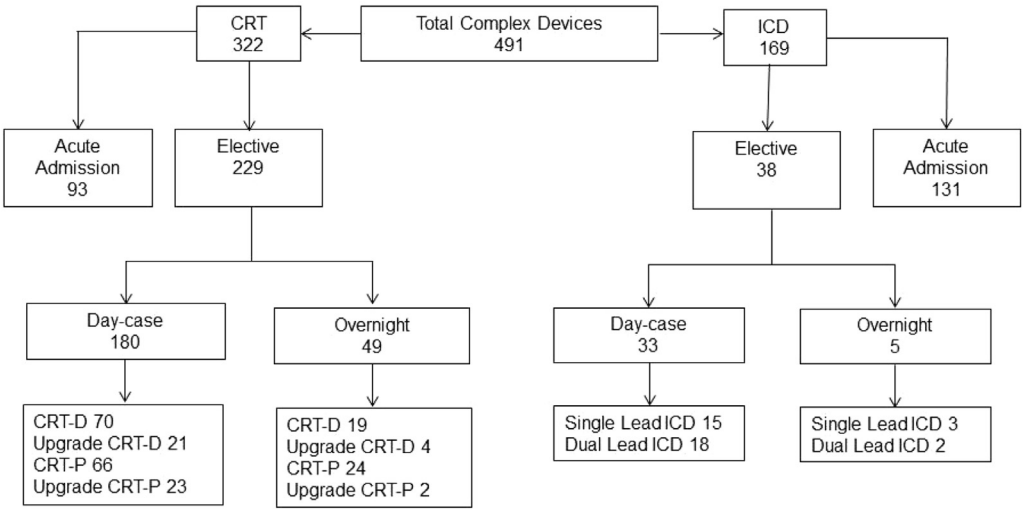

Figure 1. Figure showing the overall study cohort.

Table 1
Table showing baseline characteristics of the same-day versus overnight cohorts

Characteristics	Total Cohort (n = 267)	Same-day (n = 213)	Overnight (n= 54)	P Value
Age (yrs) median ± SD	73.0±15.0	73.0-±15.0	73.5±13.0	1.0
Men	196 (73%)	157 (74%)	39 (72%)	0.8
Device Type:				
CRT-D	114 (43%)	91 (43%)	23 (43%)	
CRT-P	115 (43%)	89 (42%)	26 (48%)	0.3
ICD	38 (14%)	33 (16%)	5 (9%)	
NYHA class				
1	23 (9%)	19 (9%)	4 (8%)	
2	21 (8%)	17 (8%)	4 (8%)	0.4
3	199 (78%)	159 (78%)	40 (76%)	
4	13(5%)	8 (4%)	5 (9%)	
Ischemic etiology	146 (56%)	115 (56%)	31 (57%)	0.9
Non-Ischemic etiology	105 (42%)	83 (43%)	22 (42%)	0.9
Atrial fibrillation/flutter	70 (26%)	56 (26%)	14 (26%)	0.96
Previous MI	117 (47%)	92 (47%)	25 (48%)	0.9
CABG	53 (21%)	43 (22%)	10 (19%)	0.7
Hypertension	113 (42%)	92 (49%)	21 (47%)	0.8
Diabetes Mellitus	57 (23%)	49 (25%)	8 (16%)	0.2
Chronic Kidney Disease	76.0 (34%)	57 (33%)	19 (37%)	0.6
Electrocardiogram: ∏				
QRS Duration				
<120msec	35 (14%)	28 (141%)	7 (14%)	
120-149msec	48 (20%)	42 (22%)	6 (12%)	
≥150msec	161 (66%)	125 (78%)	36 (22%)	0.3
LBBB morphology	144 (54%)	111 (52%)	33 (61%)	0.4
Echocardiography: ∏				
LVEF ≤35%	249 (94%)	195 (92%)	54 (100%)	0.2
Medication: ∏				
Warfarin	87 (38%)	66 (37%)	21 (43%)	0.5
Aspirin	127 (56%)	101 (57%)	26 (53%)	0.8

∏ Percentage based on available data.
NYHA = New York Heart Association; LBBB = left bundle branch block; LVEF = left ventricular ejection fraction.

LV lead through the CS. Reasons were CS occlusion (n = 2), inability to cannulate the CS because of difficult anatomy (n = 2), and no usable CS branches to place the LV lead (n = 3). Table 2 summarizes the complications rates of the 2 cohorts. There were no significant differences in the overall complication rate between the same-day versus overnight stay cohorts and no differences at any prespecified time points. The most common complication was LV lead displacement (n = 10): 3 successfully repositioned percutaneously and 7 had successful epicardial LV leads through a surgical minithoracotomy. Two developed diaphragmatic pacing immediately after operation because of LV lead microdisplacement: 1 had successful LV lead reprogramming, the other needed LV lead repositioning. One patient developed a small pneumothorax immediately after operation, which was managed conservatively and discharged home the next day. There were 4 immediate bleeding complications: 3 wound hematomas and 1 wound oozing; 3 of these patients were on warfarin (stopped preoperatively) and 1 on aspirin (not stopped preoperatively). All were treated conservatively with pressure dressings; no reintervention or blood transfusions were required. Two LV leads failed to capture at short-term follow-up (no macro-displacement on chest x-ray): 1 had reprogramming of threshold and the other required LV lead repositioning. Two patients developed wound infections (1 short-term and 1 medium-term follow-up). Both were treated successfully with antibiotics and required no further intervention. Wound site pre-erosion occurred in 1 patient at short-term follow-up and required pulse generator reburial. There were no differences in complications between the 2 cohorts. Three patients (1.1%) died at ≤30 days of device implantation; 2 from cardiovascular causes unrelated to the procedure (1 acute myocardial infarction, 1 heart failure). There were 24 patients (9.0%) who had died 1 year after device implantation; 12 from cardiovascular causes: 8 heart failure, 2 sudden cardiac death, and 2 acute myocardial infarction. No deaths were device implantation related. Two deaths had no cause identified, from primary or secondary care medical records. A cause related to device implantation was ruled out. All mortalities ≤1 year after implant occurred in CRT implants only. There were no significant differences in 30-day or 1-year mortality rates between the 2 cohorts (Table 2).

Table 2
Table showing outcomes (complications and mortality) of same-day versus ovenight cohorts

Outcomes	Total Cohort (n=267)	Same-day (n = 213)	Overnight (n=54)	P Value
Failed Procedure	7 (2.6%)	7 (3.3%)	0	0.4
Unplanned overnight stay		9 (4.3%)		
Total Complications	26 (9.7%)	20 (9.4%)	6 (11.1%)	0.8
Immediate (≤24hrs)	9 (3.4%)	7 (3.3%)	2 (3.7%)	1.0
RV Lead Displacement (n)	2	1	1	
Diaphragmatic Stimulation (n)	2	2	0	
Pneumothorax (n)	1	1	0	
Hematoma (n)	3	2	1	
Wound bleeding (n)	1	1	0	
Short term (>24hrs-6 wks)	6 (2.2%)	4 (1.9%)	2 (3.7%)	0.4
LV Lead displacement (n)	2	1	1	
RA Lead displacement (n)	1	0	1	
LV Lead not capturing (n)	1	1	0	
Wound infection (n)	1	1	0	
Pre-erosion (n)	1	1	0	
Medium term (6 wks-4 mths)	4 (1.5%)	4 (1.9%)	0 (0.0%)	0.6
LV Lead Displacement (n)	2	2	0	
RV Lead Displacement (n)	1	1	0	
Device Infection (n)	1	1	0	
Long term (>4mths)	7 (2.6%)	5 (2.3%)	2 (3.7%)	0.6
LV Lead Displacement (n)	6	5	1	
LV Lead Not Capturing (n)	1	0	1	
Mortality:				
Mortality ≤ 30 days	3 (1.1%)	2 (0.9%)	1 (1.9%)	1.0
Mortality ≤1 years	24 (9.0%)	17 (8.0%)	7 (13.0%)	0.5

There were no differences in baseline characteristics, implant data, complications, or mortality between the same-day versus overnight stay patients undergoing CRT implantation (Table 3). Vascular access for the right atrial and ventricular leads was mostly cephalic vein (70%), for LV lead was predominantly axillary vein (55%) with the remainder through the cephalic (23%) or subclavian (21%) veins. There were no differences in the axial LV lead position at the midcavity level in both cohorts (79.4% vs 69.4%, p = 0.6). There were 50 (19%) cardiac device upgrades, all of which were CRT upgrades. The original device was either a dual-chamber pacemaker (n = 25, 50%), single-chamber pacemaker (n = 6, 12%), dual-chamber ICD (n = 4, 8%), or single-chamber ICD (n = 15, 30%). There were more failed procedures in the upgraded group compared with de novo implant group (8% vs 1.2%, p = 0.04). However, there were no significant differences in complications between the 2 groups. There was a trend toward higher 1-year mortality rate in the upgrade group compared with de novo group (18% vs 9%, p = 0.07).

An overnight stay on our cardiology ward (excluding further procedures) costs $450 (£300). A similar overnight stay on our coronary care unit costs $525 (£350). Given that most stayed on our cardiology ward, we used $450 (£300) for overnight stay in our analysis. The cost was determined by the total ward budget divided by bed-days plus an allowance for supporting medical staff and services. Our same-day policy over this period resulted in 204 patients (213 planned same-day minus 9 unforeseen admissions) going home the same day. This resulted in a cost saving to our hospital of approximately $91,800 (£61,200).

Table 3
Comparison of same-day versus overnight stay CRT implantation

Characteristics	CRT total (n =229)	Same-day (n = 180)	Overnight (n=49)	P Value
Age (median ± SD)	74.0±14.0	74.5±14.0	74.0±13.0	0.4
Men	168 (73%)	134 (74%)	34 (69%)	0.5
Device type:				
CRT-D	89 (39%)	70 (39%)	19 (38%)	
Upgrade CRT-D	25 (11%)	21 (12%)	4 (8%)	0.2
CRT-P	90 (39%)	66 (37%)	24 (49%)	
Upgrade CRT-P	25 (11%)	23 (13%)	2 (5%)	
CRT-D Indication:				
Primary prevention	87 (78%)	67 (75%)	20 (87%)	0.3
Secondary prevention	25 (22%)	22 (25%)	3 (13%)	
NYHA Class				
3	192 (88%)	152 (89%)	40 (83%)	0.3
4	12 (6%)	7 (4%)	5 (10%)	
Electrocardiogram:				
QRS Duration				
120-149ms	44 (20%)	38 (22%)	6 (14%)	0.4
≥150ms	161 (75%)	125 (73%)	36 (82%)	
LBBB	142 (62%)	109 (61%)	33 (67%)	0.6
Echocardiography: ∏				
LVEF ≤35% (n, %)	225 (99%)	176 (98%)	49 (100%)	1.0
RA Lead Position: ∏				
RA Appendage	127 (56%)	97 (54%)	30 (63%)	
RA Free Wall	28 (12%)	20 (11%)	8 (17%)	0.4
No lead [due to AF]	41 (18%)	35 (20%)	6 (13%)	
Lead-in-situ	27 (12%)	23 (13%)	4 (8%)	
RV Lead Position: ∏				
RV Apex	173 (76%)	134 (75%)	39 (81%)	
RV Septum	14 (6%)	11 (6%)	3 (6%)	0.7
Lead-in-situ	37 (16%)	32 (18%)	5 (10%)	
LV Lead Position: ∏				
Anterior	15 (7%)	13 (8%)	2 (4%)	
Antero-lateral	23 (10%)	19 (11%)	4 (8%)	0.1
Lateral	129 (58%)	105 (61%)	24 (490%)	
Postero-lateral	53 (24%)	34 (20%)	19 (39%)	
Posterior	1 (0.6%)	0	1 (0.5%)	
LV Lead Displacement	10 (4.4%)	8 (4.4%)	2 (4.1%)	1.0
Mortality				
Mortality ≤ 30 days	3 (1.3%)	2 (1.1%)	1 (2.0%)	
Mortality ≤ 1year	24 (10.4%)	17 (9.4%)	7 (14.3%)	

∏ Percentage based on available data.
NYHA = New York Heart Association; LBBB = left bundle branch block; LVEF = left ventricular ejection fraction.

Discussion

Complex cardiac device implant rates continue to rise in Europe and North America with resultant increase in costs.[8,13] Complication rates for CRT/ICD implantation have repeatedly been shown to be low.[14] Several studies have demonstrated same-day ICD implantation is safe and effective but have been limited by time and specific indication.[15,16] International guidelines still do not currently recommend same-day device implantation.[13]

Complex device implantation has a low overall complication rate with high overall success for ICD (99%) and CRT (93%).[14] The rate of pneumothorax was found to be

low (0.9%), and lead displacement rates were low in ICD implants (1.8%) and tended to occur early.[14] Lead displacement was greater in CRT implants (5.9%), driven mainly by LV lead displacement (6.8%); right atrial (1.0%) and right ventricular leads (0.6%) had low displacement rates.[14] Postprocedural hematoma rates were similar for both CRT (2.4%) and ICD (2.2%) implants, although this is likely to have been underestimated. Our overall total complication rate of 9.7% was low compared with published data, and specific complication rates were within published data[14]; this was despite including 50 CRT upgrades, which are associated with higher complication rates.[12] In addition, our median follow-up of 30 months was greater than several previous CRT trials. The commonest complication in our study was LV lead displacement (4.4%) and was below the rate reported in a systematic review (6.8%).[14] Our study demonstrated that complication rates for same-day implants did not differ from those staying overnight with no differences in immediate, short-, medium-, or long-term complications and applied equally to both CRT and ICD implants.

Our study reflects real-world practice and has a high proportion of upgraded devices, specifically CRT upgrades. Patients having device upgrade have higher complication rates[12]; however, in our study, only procedure failure was greater compared with de novo CRT implants (8.0% vs 1.7%, $p = 0.04$). Upgrading simple pacemakers and ICDs to complex devices is becoming more common, although specific data on their procedural risk are limited. Poole et al.[12] demonstrated in a large multicenter prospective study of >1,700 upgraded pacemakers and ICDs over 6 months that complication rates were higher in transvenous lead replacement upgrades versus lone box changes (15.3% vs 4.0%). Hematoma formation and bleeding are common and expensive complications in terms of hospital stay.[17] The prevalence of hematoma or bleeding in our study was low (1.5%) with no difference between the same-day and overnight groups; none required reintervention or blood transfusion. Increasingly, patients have device implants on continuing oral anticoagulation. These patients may potentially require longer monitoring postoperatively because of a greater risk of bleeding. Device infection may range from superficial to deep and involve the intravascular lead.[18] Two patients in our study developed pocket infection at follow-up that was successfully treated with antibiotics; the incidence of ICD infection is estimated at 0.7% to 1.2%.[19,20] Patients most commonly present with erythema and swelling at the pocket site.[18] The time delay between device implantation and presentation with pocket infection typically ranges from 1 to 16 months after implantation.[21–23] In our study, the 2 wound or device infections occurred within the 6-week to 4-month follow-up. Three patients were kept in because of a complication (1 pneumothorax, 2 wound hematomas). No other major short-term complications were noted. Theses complications, except on rare occasions, are usually recognized and reported during or soon after the postoperative period because of their immediate hemodynamic cosequences[24]; we noted none of these following implant. Our immediate complication rate for the entire cohort was 3.4%, which is consistent with recently reported registry data.[15,25] Certain characteristics can play a major role in acute device complications such as advanced age, co-morbidity (diabetes, hypertension, renal impairment), and anticoagulant or antiplatelet therapy.[15,26] In our study, we noted 3 of 4 wound hematomas or bleeds had been on warfarin; however, none were on warfarin at implant (1 on aspirin), and all settled with conservative measurements. Significant bleeding requiring transfusion or need for pocket reexploration has been associated with use of dual-antiplatelet therapy or periprocedural heparin bridging therapy.[27] Another recent study on same-day ICD implantation demonstrated a low rate of short-term complications with 1 patient developing a delayed pneumothorax on the contralateral side.[16] However, this was a small study in patients at very low risk. Contrary to our study, they excluded patients who lived >50 miles from an emergency department and those who were pacemaker dependent; our day-case protocol had no such restrictions and included CRT implants. At a time of increasing financial pressure on the UK health care system, efficiency savings such as demonstrated are greatly welcomed. With increasing device implants seen in Europe and North America, potential cost savings with this strategy are significant.

Limitations of our study were that it was a single-center, non-randomized, retrospective study and based in a tertiary urban center. Additionally, some patients may have had minor complications (wound hematoma/superficial infection), that resolved between follow-ups and for which no medical advice was sought or been given antibiotics and not mentioned at follow-up resulting in under-reporting of minor complications. Our same-day policy started in 2010, and we were comparing patients before and after this time frame. There have been advances in implant technology during this period that could introduce a time-specific bias. Some centers still perform defibrillator threshold testing which can have implications for postprocedural monitoring and discharge. Also, cost savings for patients living in remote regions that need readmission within 24 hours may be significantly impacted.

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Disclosures

The authors have no conflicts of interest to report.

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The interplay between heart failure, metabolism and body composition

A complex interplay exists between heart failure, metabolic status and body composition. The idiosyncrasies of these relationships are poorly understood, but they offer prognostic value and potential clinical utility. Current understanding of this relationship and known clinical value are discussed in this article.

Heart failure is a common and disabling condition, defined as an abnormality in cardiac structure and/or function that is unable to meet the metabolic demands of the body. Heart failure affects 800 000 people in the UK and ultimately carries a high mortality (McMurray et al, 2012). There is strong evidence of the impact of obesity and overall body composition on the development and progression of heart failure. Understanding of this complex interplay is limited, but has clinical value given the recognized impact of adiposity and weight loss on predicting heart failure outcomes. This article summarizes the current evidence and importance of this interplay between heart failure, metabolism and body composition.

Heart failure metabolism

Under normal physiological circumstances there is a balance between anabolic and catabolic metabolism and its regulation. The development and progression of heart failure is associated with activation of neurohormonal systems, the development of a pro-inflammatory state and endothelial dysfunction (McMurray et al, 2012; Melenovsky et al, 2013; Christensen et al, 2014). The imbalance in metabolism that favours a pro-catabolic state is associated with progression of heart failure and alters skeletal and adipose tissue metabolism (Christensen et al, 2014).

Natriuretic peptides (e.g. N-terminal prohormone of brain natriuretic peptide; NT-pro-BNP) are released in response to the haemodynamic changes in heart failure and convey diagnostic and prognostic value (McMurray et al, 2012). An inverse relationship is well established between levels of natriuretic peptides and body mass index (Christensen et al, 2013). In a small cross-sectional study, Christensen et al (2014) observed that high levels of NT-pro-BNP were associated with low total fat mass (ß=-0.3, $P<0.05$).

Adipocytes are sensitive to natriuretic peptides, activating lipolysis and enhancing the expression of brown adipocyte genes; increasing energy use and thermogenesis (Christensen et al, 2014). Natriuretic peptides stimulate the release of adipokines, specifically adiponectin and leptin, which increase energy use and weight reduction (Christensen et al, 2014). Adipokines are involved in whole body energy metabolism, and adiponectin is particularly involved in the regulation of skeletal muscle metabolism and weight loss in patients with heart failure. In a cross-sectional study of elderly males with stable heart failure and no cardiac cachexia, Loncar et al (2013) observed that adiponectin was independently associated with muscle mass and strength. In a pivotal prospective observational study of right ventricular dysfunction and cardiac cachexia (n=408), Melenovsky et al (2013) identified that adiponectin levels were significantly raised in both patients with right ventricular dysfunction who were cachectic. Furthermore, adiponectin was one of the few variables (alongside NT-pro-BNP, right ventricular dysfunction and neurohormonal antagonist therapy) to independently predict cardiac cachexia (Melenovsky et al, 2013).

Serum adiponectin levels are associated with severity of heart failure and adverse outcomes (Loncar et al, 2013). Paradoxically, adiponectin has been observed to have beneficial effects on lipid and glucose metabolism, alongside myocardial inflammation, hypertrophy and

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fibrosis (Okamoto, 2009). It has been identified as a well-placed potential biomarker for the cross-talk in heart failure metabolism (Okamoto, 2009).

Pro-inflammatory signals from cytokines and interleukin-6 (IL-6) are increased in patients with heart failure (Christensen et al, 2014). Proteolysis in muscle occurs predominantly via the ubiquitin–proteasome system, which has increased activation in patients with heart failure as a result of stimulation from these increased pro-inflammatory signals (Fülster et al, 2013). Christensen et al (2014) described a trend towards an association between high IL-6 levels and fat free mass. Adiponectin and leptin have receptors in skeletal muscle which have acute and chronic effects on local metabolism (Loncar et al, 2013).

Adiposity and heart failure

Obesity, defined as a raised body mass index (>30.0 kg/m^2), is recognized as a risk factor for heart failure. The risk of developing heart failure increases for men and women by 5% and 7% respectively for every one unit rise in body mass index, independent of other important co-variables (Clark et al, 2014). A graded increase in risk of developing heart failure is recognized for increasing body mass index in both males and females in different population groups (Clark et al, 2014). Furthermore, Clark et al (2014) describe the increased risk of heart failure from other raised adiposity surrogate metrics, for example waist circumference and waist–hip ratio.

Counterintuitively, those with a raised body mass index and established heart failure have been observed to have improved outcomes (Oreopoulos et al, 2008; Pocock et al, 2008; Futter et al, 2011; Clark et al, 2014). In a large meta-analysis of 28 209 patients with heart failure who were obese or overweight (25.0–29.9 kg/m^2), Oreopoulos et al (2008) found an all-cause mortality of -19.0% and -40.0% and cardiovascular mortality of -16.0% and -33.0% respectively compared to those without a raised body mass index (<24.9 kg/m^2) at >2years follow-up. The relationship between body mass index and mortality has a U-shaped curve with the lowest rates associated with those overweight and obese and the higher rates associated with leanness and severe obesity (>35.0 kg/m^2) (Pocock et al, 2008), although not all datasets have replicated this finding (Futter et al, 2011).

The inverse relationship of NT-pro-BNP and adiponectin with body mass index and total percentage body fat suggests that a higher fat content protects against the catabolic activity of these neurohormonal signalling pathways, and supports the observation that fat mass is preserved in patients with cardiac cachexia (Christensen et al, 2013; Loncar et al, 2013). In a retrospective cohort study of 219 Chinese patients with severe left ventricular systolic dysfunction (ejection fraction <35%), Cai et al (2014) demonstrated that overweight (24.0–28.0 kg/m^2) and obese (>28.0 kg/m^2) predicted response to cardiac resynchronization therapy and improved survival at 6 months. Notably in this study the defined body mass index ranges were lower than those used in other studies because the Chinese population has a lower average body mass index than western populations. Furthermore, this study demonstrated that the obese population better tolerated optimal medical therapy, an observation noted in other studies (Melenovsky et al, 2013; Cai et al, 2014).

> **“A graded increase in risk of developing heart failure is recognized for increasing body mass index in both males and females in different population groups.”**

The paradoxical observations that an overweight or obese body mass index predicts development of heart failure but offers improved survival once established is referred to as the ‘obesity paradox’ (Pocock et al, 2008; Clark et al, 2014). Several explanations and hypotheses have been offered to explain the obesity paradox. First, lower levels of natriuretic peptides are seen in obese patients, therefore symptoms may present earlier (Clark et al, 2014). Second, patients with lower levels of circulating natriuretic peptides are thought to have a more attenuated renin–angiotensin–aldosterone system (Clark et al, 2014). Despite this, obesity also contributes to maintaining systemic blood pressure, preserving renal function, which allows patients to better tolerate anti-heart failure medication (Clark et al, 2014). Third, body mass index is a crude metric of body composition, with some patients classified as overweight or obese actually containing a high proportion of muscle; body mass index does not account for the different body composition components (Clark et al, 2014). Finally, obesity is a heterogeneous condition with various fat mass distributions. These can include visceral fat deposits or subcutaneous or gluteofemoral obesity, each of which have differing metabolic profiles (Clark et al, 2014).

Body composition and heart failure

Muscle wasting is common in patients with heart failure. Sarcopenia, defined as reduced muscle mass and limited mobility, occurs naturally with aging at a rate of 1–2% per annum over 50 years of age. Fülster et al (2013) recruited 200 patients with heart failure at a single centre and observed that 19.5% of the cohort had sarcopenia, a higher proportion than would be expected through natural aging. Significantly patients with heart failure with both reduced (68.8%) and preserved (31.2%) ejection failure were recruited to this study (Fülster et al, 2013). Heart failure patients with sarcopenia had a higher incidence of reduced left ventricular ejection fractions, reduced muscle strength, worse functional capacity and significantly higher IL-6 levels (Fülster et al, 2013). Elevated levels of pro-inflammatory signals in patients with heart failure, including cytokines and IL-6, stimulate catabolic pathways, for example the ubiquitine-protease pathway, and cause sarcopenia (Fülster et al,

Long-Term Follow-Up of Isolated Epicardial Left Ventricular Lead Implant Using a Minithoracotomy Approach for Cardiac Resynchronization Therapy

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Background: *Transvenous left ventricular (LV) lead placement for cardiac resynchronization therapy is unsuccessful in 5–10% of reported cases. These patients may benefit from isolated surgical placement of an epicardial LV lead via minithoracotomy approach.*

Aim: *To evaluate the success of this approach at long-term follow-up.*

Methods: *Retrospective evaluation of all consecutive patients undergoing isolated epicardial LV lead placement after failed transvenous attempt over a 6-year period. Data collected on baseline parameters, procedural details, and outcome at follow-up (hospital stay, complications, mortality, and clinical response).*

Results: *Forty-two patients underwent epicardial lead implant. Five died within 1 year (11.9%): two (4.8%) died within 30-days post op (one from intraoperative hemorrhage, the other from multiple organ failure); 39 (95.1%) were admitted to the high dependency unit and transferred to the ward <24 hours. Median hospital stay was 3.4 ± 1.9 days. The overall complication rate was 17.5% (n = 7): 15.0% (n = 6) short term and 2.5% (n = 1) long term; these included three (7.5%) LV noncapture events all treated with reprogramming. There were two (5.0%) wound infections requiring oral antibiotics and two (5.0%) device infections requiring intravenous antibiotics (one had device resiting, the other developed septic shock requiring intensive care admission). Assessment of clinical response was possible in 34 (81.0%) at follow-up: 21 (61.8%) were responders and 13 (28.2%) nonresponders with no significant differences between these groups; no clinical predictors of response were identified.*

Conclusion: *Isolated epicardial LV lead implant using minithoracotomy is relatively safe and effective at successful LV pacing. Response rate and postoperative recovery at long-term follow-up are reasonable in these high-risk patients. (PACE 2016; 39:1052–1060)*

cardiac resynchronization therapy, epicardial LV lead, minithoracotomy

Introduction

Cardiac resynchronization therapy (CRT) is a highly successful treatment option for chronic heart failure with cardiac dyssynchrony that is refractory to medical therapy.[1,2] Successful placement of the left ventricular (LV) lead is essential in order to achieve resynchronization. The initial approach is via the transvenous route utilizing the coronary sinus. However, LV lead placement at an optimal site can be challenging due to anatomical or pathological factors (such as the presence of myocardial scar). Failure of LV lead delivery via the transvenous route has been reported in 5–10% of cases.[3–5] Epicardial LV lead placement is an important second-line option to allow these patients to benefit from CRT.[4,5] Current national and international guidelines on CRT have resulted in ever-increasing numbers of patients being offered this therapy, resulting in a higher demand for alternative LV lead placement options if the transvenous route fails.[6]

Multiple surgical options are available for placement of an epicardial LV lead. These include use of a median sternotomy (such as with coronary artery bypass grafting [CABG] or

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valve repair/replacement), full left lateral thoracotomy, and a minimal thoracotomy approach (minithoracotomy).[5,7] The full lateral thoracotomy offers wider access to the left lateral wall of the left ventricle. However, the minithoracotomy approach is less invasive and is used more commonly because of the lower incidence of mediastinitis or osteomyelitis.[8] The reported success of surgically placed epicardial LV leads is variable, in terms of durability and complications, both in the short term and long term.[3,9–11] Long-term infection of devices has been quoted to be as high as 9.6%.[9] Nonresponse among heart failure patients undergoing CRT represents a significant problem and has been reported to occur in up to 30% of implants.[1] Mechanical and anatomical causes can contribute to this burden.

The objective of this study was to report our 6-year experience of the minithoracotomy approach for isolated placement of epicardial LV leads following failure of the traditional transvenous route. We report the success of this approach, length of hospital stay, complications, and clinical predictors of response following epicardial LV lead placement.

Methods

Study Design

We performed a retrospective study of all consecutive patients who underwent surgical placement of an epicardial LV lead via a minithoracotomy approach at University Hospital Coventry, UK, between November 2007 and November 2013. Eligibility for the study was that all patients had at least one attempt at transvenous LV lead implant via the coronary sinus as part of a CRT-pacemaker or CRT-defibrillator (CRT-D) implant. All patients met national criteria for CRT implantation and both *de novo* and upgraded cardiac devices were included in the study. All surgical epicardial LV lead placements using alternative approaches were excluded. Patient electronic and paper case records were used to obtain baseline patient demographics, procedural details, length of hospital stay, epicardial lead data, and outcome measures. Approval for the study was obtained from our hospital's Research, Development, and Innovation department. The study conformed to the declaration of Helsinki.

Approach to Performing Minithoracotomy

All procedures were performed in the operating theater under general anesthesia on the beating heart by one of two experienced cardiothoracic surgeons (WD, SKB); both surgeons had extensive cardiac surgical experience as consultants (WD 30 years/SKB 10 years) and each had comprehensive experience of the technique. The incision was left submammary with standard monitoring in all cases (electrocardiogram, pulse oximetry, invasive arterial monitoring, central venous line, and external defibrillator pads) and Swan-Ganz catheter used if needed. Standard single lumen intubation was utilized. Patients were placed in a supine position with the left chest elevated by 30°–40°. A 3–4-cm incision was made in the left submammary region to enter either the 4th or the 5th intercostal space depending on the chest x-ray. The pericardial fat was dissected superiorly and the pericardium opened vertically using a 1–2-cm incision. The lateral LV wall of heart was identified and the muscular portion approached for implantation of a screw-on unipolar lead (Medtronic 5071, Medtronic Inc., Minneapolis, MN, USA) in all cases. Lead threshold measurements were taken and data accepted if threshold was <2 V and R-wave sensing >4 mV. The IS-1 connector of the lead was passed through the same intercostal space and tunneled submuscular to a left pectoral pocket and the pacemaker pulse generator connected. The incisions were closed in a layered manner.

Device Follow-Up

Each patient was seen routinely following LV epicardial lead placement by the cardiology/cardiothoracic team and at the CRT pacing clinic. Referrals from external centers were often repatriated for continued care and follow-up data obtained from those centers. Following LV epicardial lead placement, routine device check was performed initially at 2 months, 6 months, and then annually, dependent on the local center policy and individual clinical situation. Epicardial LV lead data (impedance and threshold) were recorded following the first check and annual check.

Outcomes Measures

The outcome measures reported included hospital stay, complication rates, and mortality at follow-up. Duration of stay on the high dependency unit (HDU) and overall hospital stay was reported for each patient in the study. The overall complication rates were recorded and defined as immediate (≤24 hours), short term (>24 hours to ≤4 months), and long term (>4 months to 1 year). Complications were broadly classified as failure of procedure, epicardial LV lead failure, infection, and hemorrhage (defined

as >2 g/dL hemoglobin reduction requiring blood transfusion). All-cause mortality was recorded for all patients up to 1-year postprocedure. The 30-day mortality rate was examined separately from complications.

Clinical Response

Clinical response was defined as a reduction of New York Heart Association (NYHA) class ≥1. NYHA symptom classification was determined based on the first formal assessment following the procedure. Clear documentation of NYHA score or detailed symptom evaluation was required for an independent reviewer to conclude NYHA score. Where classification was unclear, a second reviewer assessed the consultation, requiring a consensus to determine a score. Where no clear documentation or consensus was achievable, then no response classification was possible.

Statistical Analysis

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS), version 22.0 (IBM Corp., Armonk, NY, USA). Categorical variables were reported as frequency and percentages. Comparison analyses for categorical data were performed using the χ^2 and Fisher's exact tests. Continuous data underwent histogram plots for assessment of normality. Normally distributed data were reported as mean ± standard deviation (SD) and comparative analysis was performed using independent *t*-tests. Nonnormally distributed data were reported as median with interquartile range and were compared using a Mann-Whitney U test. Paired continuous data (dependent on whether distribution parametric or nonparametric) were compared with a paired *t*-test or Wilcoxon signed-rank test, respectively. Univariate logistic regression analysis was performed on clinical response. Those variables that achieved a P value <0.15 were pooled as covariants for multiple logistic regression. A high alpha was set on the basis of the clinical response definition. A stepwise entry method was applied with forward selection and backward elimination to ensure duplication of findings. The accuracy of the model was verified with a Hosmer-Lemeshow goodness-of-fit test. A P value <0.05 was considered statistically significant.

Results

A total of 42 patients underwent epicardial LV lead placement using the minithoracotomy approach. Table I demonstrates the baseline characteristics of the cohort. The reason for referral for epicardial LV lead placement is shown in Figure 1. A total of 41 (97.6%) patients had successful LV lead placement, with one mortality occurring intraoperatively. All LV lead data at implant were accepted if LV threshold was ≤2.0 V with R-wave sensing >4 mV; this was noted in all LV lead implants. No patients required cardiopulmonary bypass. Six (14.3%) patients had an additional procedure performed at the same time (four had pulse generator changes, one resitting of pulse generator pocket, and one removal of transvenous LV lead). None of these additional procedures were associated with any complication or 30-day mortality. Overall success rate was the same for both surgeons and overall individual complication rate was not statistically different (13.0% vs 21.1%, P = 0.78).

Table I.

Baseline Characteristics of Overall Cohort

Baseline Characteristics	N = 42
Age (median and IQR)	73.5 (64.0–76.3)
Male (n, %)	33 (78.6%)
CRT-D implant (n, %)	21 (50.0%)
CRT upgrade (n, %)	10 (23.8%)
NYHA baseline (mean ± SD)	2.95 ± 0.38
Ischemic cardiomyopathy (n, %)	29 (69.0%)
Myocardial infarction (n, %)	23 (54.8%)
Previous PCI (n, %)	10 (23.8%)
Previous CABG (n, %)	10 (23.8%)
Diabetes mellitus (n, %)	12 (28.6%)
Hypertension (n, %)	25 (59.5%)
Chronic kidney disease (n, %)	13 (31.0%)

CABG = coronary artery bypass grafting; CRT = cardiac resynchronization therapy; CRT-D = CRT-defibrillator; IQR = interquartile range; NYHA = New York Heart Association; PCI = percutaneous coronary intervention; SD = standard deviation of mean.

Hospital Stay

Postoperatively all patients were transferred to the HDU, with the exception of one patient who was transferred directly to the ward. A total of 39 (95.1%) patients spent 1 day on HDU before being transferred to the ward. The remaining patients spent 7 days on HDU before dying from multiple organ failure. Total hospital stay for patients discharged (n = 40) was 3.4 ± 1.9 days. Four patients were transferred back to the referring center for convalescence with no prolonged stay reported at the referring centers.

Complications

The total number of complications following the procedure within 1 year was nine (21.4%); this included two patients who died within the

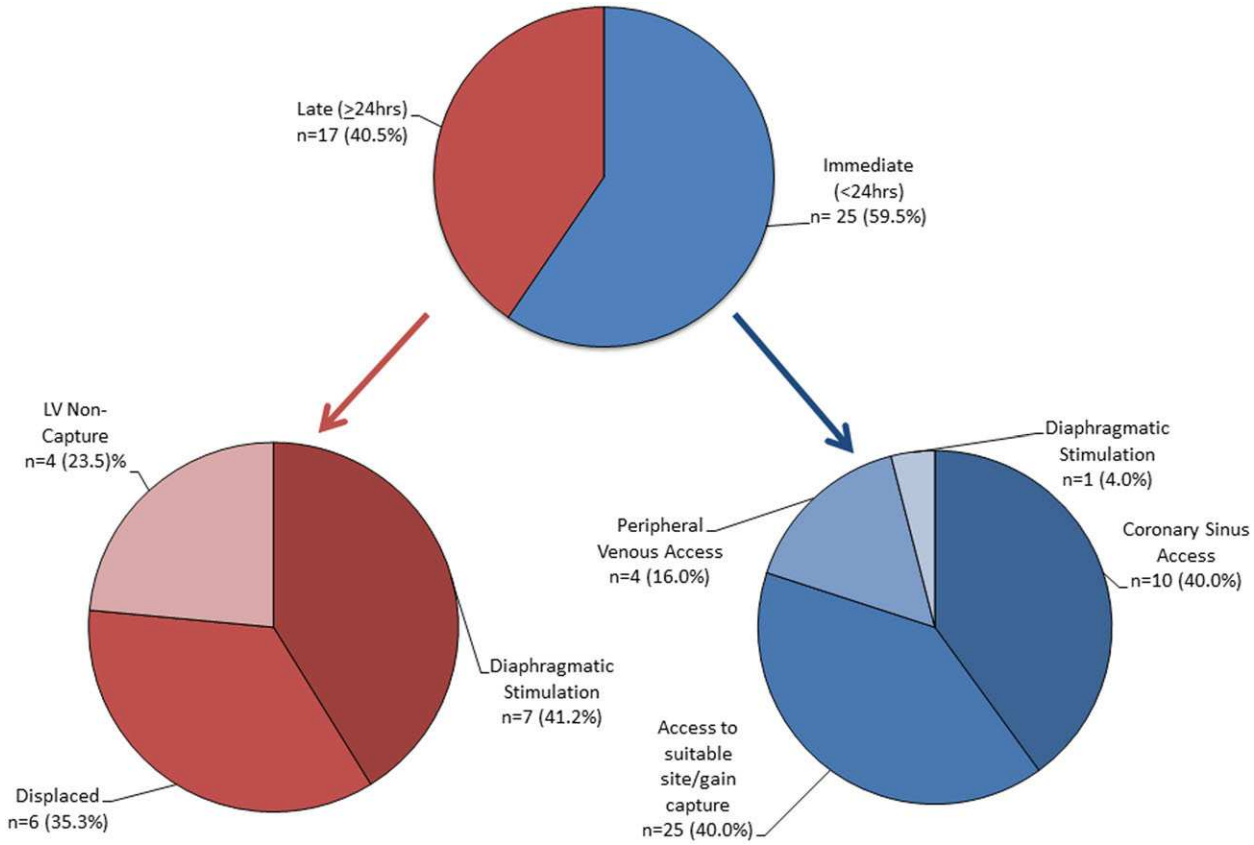

Figure 1. *Transvenous left ventricular lead failure reasons (N = 42).*

Table II.

Overall Complications, Both Short Term and Long Term (N = 40)

Short Term (>24 Hours to ≤4 Months) n = 6 (15.0%)		**Long Term (>4 Months to 1 Year) n = 1 (2.5%)**	
LV lead noncapture (n, %)	2 (5.0%)	LV lead noncapture (n, %)	1 (2.5%)
Wound infection (n, %)	2 (5.0%)		
Device infection (n, %)	2 (5.0%)		

LV = left ventricular.

hospital admission (discussed separately later). The number of complications of those discharged from hospital (n = 40) was seven (17.5%). Table II shows these specific complications classified by the predefined time period postprocedure. There were three LV lead noncapture events at follow-up with an initial attempt at reprogramming made in all; this was successful in one patient. The second patient went on to have a reattempt at transvenous LV lead placement which failed; the patient was referred back for a repeat LV epicardial lead placement, but died waiting for the lead placement from unrelated events over a year after the initial implant. The remaining patient did not want any further intervention and went on to have the CRT removed and leads capped.

Infection occurred in four (10%) patients within a year of the epicardial lead placement. Wound infection occurred in two of these cases and was treated successfully with one course of oral antibiotics. Device infection accounted for the other two patients and both required hospital admission; one patient developed septic shock secondary to his device infection and was

treated with intravenous antibiotics; there was no evidence of infection on the intravascular leads. The patient required admission to the intensive care unit for inotropic support. He responded well to medical therapy and did not require further intervention on his device. The remaining patient required intravenous antibiotics and resiting of the CRT pulse generator. All patients made a full recovery from these episodes.

Upgrade versus Nonupgrade Cases

There were 32 *de novo* and 10 upgrade CRT cases. All *de novo* epicardial LV lead placements were successful compared with nine upgraded CRT cases. The one patient who was not successful had the intraoperative mortality and was originally a CRT-D upgrade. In the *de novo* group, there were four (12.4%) complications and in the upgrade group, there were three (30.0%) complications with no statistical difference noted (P = 0.42). The specific complications that occurred for CRT upgrade cases were one wound infection, one device infection (requiring intravenous antibiotics and device resitting), and one LV lead noncapture. No difference in complication pattern was observed between both groups.

Epicardial LV Lead Data

There were 26 patients who had epicardial LV lead data available at the first checkup (median 2.1 [1.2–3.0] months). Four deaths occurred before the first routine device checkup. One patient was immediately transferred to an external center outside the region. There were 13 patients whose epicardial LV lead data were not available despite extensive searches at several centers. The annual review epicardial LV lead data were available for 23 patients; two patients were transferred to external centers and one patient died following the first checkup (median 13.3 [11.7–14.2] months). Figure 2 shows there was no significant change in epicardial LV lead threshold or impedance between the two checks.

Mortality

There were five (11.9%) deaths within 1 year of the epicardial procedure. The median time to 1-year mortality was 1.3 (0.1–4.1) months. Two patients (4.8%) died within 30 days of the procedure: one died intraoperatively due to catastrophic hemorrhage from a left atrial appendage rupture. Despite operative intervention and aggressive resuscitation, the bleeding site extended and the situation was unfortunately irreversible. The other patient died postoperatively on HDU 7 days after the procedure. The procedure was complicated by one episode of ventricular tachycardia upon identifying and opening the pericardium; the patient had a background of severely impaired LV systolic function due to dilated cardiomyopathy. This was treated immediately by direct current cardioversion and administration of adrenaline. The remaining part of the procedure was uncomplicated and the patient remained hemodynamically stable. The patient was transferred to the HDU for more intensive monitoring following the ventricular tachycardia. Initially the patient recovered well; however, he developed paralytic ileus followed by acute on chronic renal failure and multiple organ failure. Vasopressor drug requirements continued to increase without any improvement in clinical course. The decision was taken that the patient was unlikely to survive and was made comfortable. The patient was at high surgical risk prior to the procedure being performed given his comorbidity (severe heart failure and chronic renal impairment). Three further patients had died by 1-year postprocedure; none of these deaths were attributable to the placement of the epicardial LV lead.

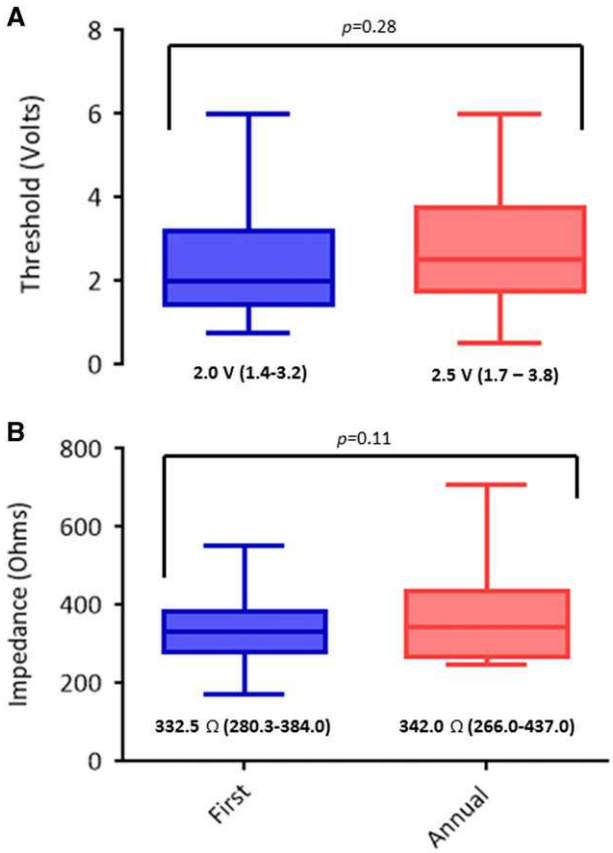

Figure 2. *Epicardial left ventricular lead data (threshold @ 1 ms and lead impedance) between first (N = 26) and annual device check (N = 23).*

Table III.

Responder and Nonresponder Baseline Characteristics (N = 34)

	Response n = 21 (61.8%)	Nonresponse n = 13 (38.2%)	P Value
Age (median and IQR)	71.0 (61.5–76.0)	72.0 (65.5–77.0)	0.52
Male (n, %)	17 (81.0%)	9 (69.2%)	0.71
CRT-D (n, %)	11 (52.4%)	6 (46.2%)	1.00
Upgrade (n, %)	6 (28.6%)	2 (15.4%)	0.64
NYHA baseline (mean ± SD)	3.0 ± 0.45	2.9 ± 0.38	0.31
NYHA follow-up (mean ± SD)	1.62 ± 0.67	2.9 ± 0.38	0.00
Ischemic cardiomyopathy (n, %)	14 (66.7%)	9 (69.2%)	1.00
Myocardial infarction (n, %)	9 (42.9%)	9 (69.2%)	0.25
Angina (n, %)	8 (38.1%)	3 (23.1%)	0.59
Previous PCI (n, %)	4 (19.0%)	4 (30.8%)	0.71
Previous CABG (n, %)	3 (14.3%)	6 (46.2%)	0.1
Diabetes mellitus (n, %)	7 (33.3%)	2 (15.4%)	0.45
Hypertension (n, %)	12 (57.1%)	7 (53.8%)	1.00
Chronic kidney disease (n, %)	5 (23.8%)	4 (30.8%)	0.96
Complication 1 year (n, %)	6 (28.6%)	1 (38.2%)	0.305

Abbreviations as in previous tables.

Table IV.

Logistic Regression Analysis for Clinical Predictors of CRT Response

	Univariate			Multivariate		
	OR	P Value	CI	OR	P Value	CI
Age	0.96	0.39	0.88–1.05			
Male	1.89	0.44	0.38–9.40			
CRT-D	1.28	0.72	0.32–5.13			
Upgrade	2.20	0.39	0.37–13.04			
Ischemic cardiomyopathy	1.13	0.87	0.25–5.00			
Myocardial infarction	0.33	0.14	0.07–1.44	0.46	0.33	0.10–2.17
Previous PCI	0.53	0.44	0.11–2.63			
Previous CABG	0.19	0.05	0.038–1.00	0.24	0.10	0.04–1.32
Diabetes mellitus	2.75	0.26	0.47–16.0			
Hypertension	0.88	0.85	0.21–3.51			
Chronic kidney disease	0.70	0.65	0.15–3.31			
Complication 1 year	4.80	0.17	0.51–45.5			

CI = confidence interval; OR = odds ratio. Other abbreviations as in previous tables.

Clinical Response

The clinical response status of the cohort was definable in 34 (81.0%) patients. Two patients died before any formal follow-up could be performed; a further six patients did not have a follow-up NYHA classification performed or definable. Table III compares clinical variables between responders and nonresponders. The median time to first cardiology/cardiothoracic surgery follow-up was 7.3 weeks (range 0.6–61). Specifically, the median follow-up for responders and nonresponders was 7.9 weeks (range 3.0–52.1) versus 6.0 weeks (range 0.6–61.0 weeks), P = 0.1. Table IV demonstrates logistic regression univariate and multivariate analysis. Baseline NYHA classification was not used in the analysis as it was used to define the clinical response outcome measure. The multivariate analysis was verified by the Hosmer-Lemeshow goodness-of-fit test (P = 0.144). Significant independence

of predictors to determine clinical response following epicardial LV lead insertion was not demonstrated, but a trend toward a worse outcome was seen with previous CABG. The presence of any previous complication demonstrated no difference between clinical responders and non-responders.

Discussion

Isolated surgical epicardial LV lead placement is well established as a second-line option for failed transvenous LV lead placement during CRT and is increasingly being utilized.[5] The failure rate of the transvenous route for LV lead implant has been reported between 5% and 10%.[5] Placement of a transvenous lead can be suboptimal due to coronary venous anatomy and presence of myocardial scar burden.[5,12] Furthermore, complications can occur with the LV lead with the most frequent displacement at 6.8%.[13] Occasionally, these complications cannot be corrected percutaneously and require surgical placement. Our cohort represents a large regional center for performing surgical epicardial lead placements over 6 years and the commonest reason for referral was failure to percutaneously place the LV lead in the coronary sinus at CRT implant in 59.5%. The commonest reason for failure was the inability to access the coronary sinus branch or achieve adequate pacing capture. Late failure of the transvenous LV lead accounted for referral in 40.5% of cases, which was most common for diaphragmatic stimulation.

The surgical approach to epicardial lead placement offers several advantages over the transvenous approach. Overall, the surgical approach has a lower risk of lead dislodgement and phrenic nerve stimulation.[11] Moreover, placement of the LV lead is not limited by coronary venous anatomy.[14] Despite these advantages, there are several important disadvantages to the approach. The general anesthesia risk is significant in a high-risk heart failure group.[5] The approach is more invasive and can be limited by adhesions and epicardial fat.[5] Furthermore, the recovery period, which usually involves a HDU stay, is significantly more prolonged with a surgical approach.[11]

Miller et al. suggested a significantly higher mortality in patients having isolated surgical LV lead placement compared with transvenous lead placement within the first 3 months postprocedure.[15] In contrast, Patwala et al. demonstrated a reasonable success in the 3–6 months following placement.[10] The failure rate for epicardial leads has been reported to become higher and more consistent the further away from the procedure the patient moves.[5] The superiority of transvenous LV lead placement at the initial procedure is clear, but the surgical approach remains the main second-line option.

Numerous surgical approaches are available to implant an epicardial LV lead. A full left thoracotomy approach is used primarily for CABG and/or valve repair/replacement. This approach offers the widest access to the LV lateral wall, but is used less frequently for isolated lead placement due to the prolonged recovery and potential risks.[5] The minithoracotomy approach is a common method used by many centers due to improved survival and reduced incidence of mediastinitis or osteomyelitis.[3,8] The procedure demonstrated long-term hemodynamic benefit, improved LV ejection fraction, and NYHA score that can be achieved following an LV epicardial lead placement via a minithoracotomy approach.[16] Video-assisted thoracoscopy offers direct visual control and precise delivery of the epicardial lead tip.[5] The procedure offers fewer incisions and is often better tolerated, though specialists and specific technology is required.[5] The novel surgical option of robotic assisted surgery is available and boasts a 98% technical success rate and low complication rate.[17] The greatest restriction to the use of this technology is cost, though extensive follow-up data are not available to judge the success of the therapy.[5]

Isolated surgical delivery of an epicardial LV lead is commonly delivered by the minithoracotomy approach when the transvenous route is not possible or has failed. Several studies have demonstrated variable success of the minithoracotomy approach for isolated LV lead placement.[5,10,11,15] Our cohort offers a large single tertiary center long-term experience of isolated surgical placement of epicardial LV leads via the minithoracotomy approach. The 1-year all-cause mortality rate for our cohort was 11.9%, which is lower than reported in other studies.[15] In our cohort, 4.8% died while in the hospital following the procedure, including one patient who died intraoperatively from a significant bleed. Mair et al. reported a 30-day mortality of 6.3%, though this relates to one death that was not directly attributed to the minithoracotomy procedure.[3] In the cohort, 95% were discharged from the intensive therapy unit within 24 hours and one patient went straight from surgical recovery to the step-down ward, which is lower than reported by Doll et al. at 3.8 days.[11] Our overall complication rate was similar to those reported previously.[3,9] Infection postprocedure was the commonest complication, accounting for 10% of the cohort, which is comparable to other reported infection rates,[9] all occurring at short-term follow-up. Half were wound infections not requiring hospitalization; the other half were device infections requiring

Cardiac resynchronization therapy and its role in the management of heart failure

ABSTRACT

The prevalence of heart failure is increasing and it is associated with significant mortality and morbidity. Optimal medical therapy improves outcome, but heart failure continues to have a substantial impact on both the individual patient and wider society. Over the last two decades, cardiac resynchronization therapy has revolutionized the treatment of selected patients who have heart failure. Cardiac resynchronization therapy significantly reduces mortality and hospitalization through reverse cardiac remodelling. This review informs non-specialists about cardiac resynchronization therapy and for which patients it should be considered.

The burden of heart failure on both the individual patient and wider society continues to increase despite optimal medical therapy. Cardiac resynchronization therapy has become established therapy for selected patients with heart failure who are refractory to optimal medical therapy.

Heart failure is defined as an abnormality in cardiac structure and function that leads to the inability of the heart to deliver adequate levels of oxygen to match the metabolic demand of the tissues (McMurray et al, 2012). Patients commonly suffer from a plethora of symptoms including breathlessness, ankle oedema and fatigue (McMurray et al, 2012). Heart failure affects approximately 800 000 people in the UK (McMurray et al, 2012), about half of whom have heart failure with reduced ejection fraction. The mortality from heart failure is estimated at 30–40% within a year of diagnosis (Cowie et al, 2000), a rate that surpasses that of many malignancies. Several pharmacological agents, specifically angiotensin-receptor blockers (Swedberg and Kjekshus, 1988), beta-blockers (Packer et al, 1996), mineralocorticoid receptor antagonists (Pitt et al, 1999) and angiotensin receptor neprilysin inhibitors (Packer et al, 2015), significantly improve morbidity and mortality. Despite advances in medications the incidence and prevalence of heart failure continues to rise and confers a poor prognosis (McMurray et al, 2012). Cardiac resynchronization therapy has revolutionized management of patients with heart failure and improved outcomes.

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Heart failure with reduced ejection fraction and cardiac dyssynchrony

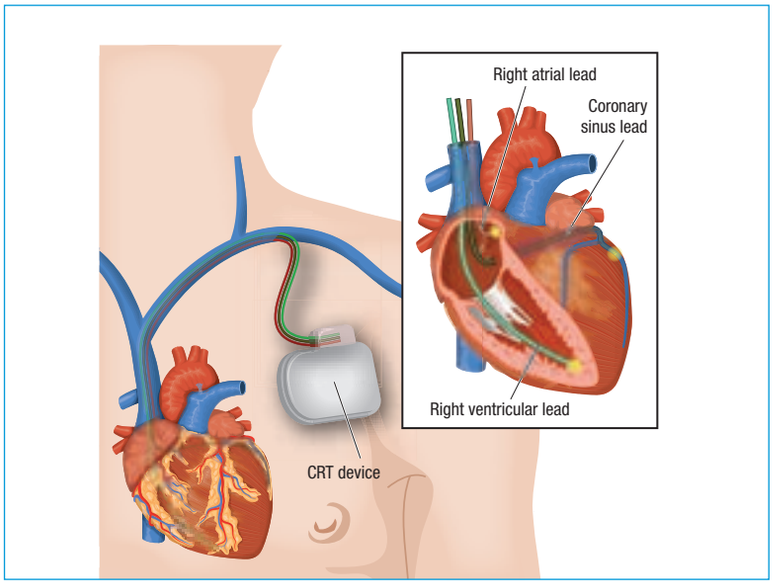
Many patients with heart failure with reduced ejection fraction develop dyssynchronous contractions of the heart as a result of damage to the underlying conduction tissue causing inefficient cardiac contraction that leads to symptoms. Cardiac dyssynchrony is a complex and multifactorial process that impacts function (Brignole et al, 2013). Prolongation of atrioventricular conduction encroaches on the starting of systole and filling of early diastole. With delayed ventricular contraction, the left ventricular diastolic pressure exceeds left atrial pressure during passive filling, leading to development of functional mitral regurgitation (Brignole et al, 2013). The impact of reducing ventricular pre-load leads to reduced left ventricular contractility, by the Starling mechanism. Moreover, the occurrence of intra- and inter-ventricular conduction delay causes asynchronous left ventricular contraction (so-called mechanical dyssynchrony) leading to reduced stroke volume, left ventricular ejection fraction and systolic blood pressure (Brignole et al, 2013). Ventricular dyssynchrony leads to dis-coordinated papillary muscle contraction and further contributes to development and progression of functional mitral regurgitation, with the whole process contributing to left ventricular adverse remodelling (Brignole et al, 2013).

Cardiac resynchronization therapy

Cardiac resynchronization therapy or 'biventricular pacing' involves implanting pacing leads into the heart via the transvenous route to the right and left ventricles (the latter through the coronary sinus) to resynchronize ventricular contraction. A lead is implanted to the right atrium to achieve atrioventricular synchrony (*Figure 1*) unless the patient has permanent atrial fibrillation. Cardiac resynchronization therapy can 'resynchronize' cardiac contraction through restoration of inter-/intra-ventricular and atrioventricular dyssynchrony (Brignole et

al, 2013). Cardiac resynchronization therapy improves left ventricular ejection fraction, left ventricular contractility, left ventricular filling time and reduces functional mitral regurgitation, which in turn can induce reverse left ventricular remodelling (Brignole et al, 2013). Cardiac resynchronization therapy can also be combined with a defibrillator function in selected patients. Implantable cardioverter defibrillators are designed to reduce the risk of sudden cardiac death caused by ventricular arrhythmias, by delivering anti-tachycardia pacing or shock therapy. This can be performed for those identified at high risk (primary prevention) or those who have had a malignant ventricular arrhythmia and survived (secondary prevention) (Goldenberg et al, 2011). Cardiac resynchronization therapy devices that have defibrillator functionality are referred to as cardiac resynchronization therapy-defibrillators and those without as cardiac resynchronization therapy-pacemakers.

Figure 1. Cardiac resynchronization therapy (CRT) device.



Who benefits from cardiac resynchronization therapy?

Over the last 20 years cardiac resynchronization therapy has become one of the most effective treatments for heart failure and is appropriate for ~25–30% of heart failure patients (Daubert et al, 2012). Cazeau et al (1994) showed that a cardiac resynchronization therapy-pacemaker in a 54-year-old patient with advancing heart failure improved New York Heart Association (NYHA) symptoms. Since then multiple randomized controlled trials have demonstrated the resounding benefit of cardiac resynchronization therapy for patients with heart failure with reduced ejection fraction who have mechanical dyssynchrony, with reduced mortality and hospitalization (Bristow et al, 2004; Cleland et al, 2005) alongside improved quality of life (Cleland et al, 2005; Moss et al, 2009; Tang et al, 2010), symptoms (Abraham et al, 2002), functional performance (Abraham et al, 2002) and left ventricular volumes (Linde et al, 2008). *Table 1* summarizes the largest randomized controlled trials examining cardiac resynchronization therapy in specific patients with heart failure with reduced ejection fraction

Table 1. Randomized control trials evaluating cardiac resynchronization therapy in sinus rhythm

Trial (reference)	No	Study design	Inclusion	Outcome	Main findings
COMPANION (Bristow et al, 2004)	1520	Double-blinded, randomized – optimal medical therapy *vs* CRT-d or CRT-p, 15 months	NYHA III–IV, left ventricular ejection fraction <35%, QRS >120 msec	Primary – all-cause mortality or hospitalizations, secondary – all-cause mortality, cardiac mortality	CRT-d and CRT-p reduced all-cause mortality and hospitalization
CARE-HF (Cleland et al, 2005)	813	Double-blinded randomized – optimal medical therapy *vs* CRT-p, 29.4 months	NYHA III–IV, left ventricular ejection fraction <35%, QRS >120 msec	Primary – all-cause mortality or hospitalizations, secondary – all-cause mortality, NYHA, quality of life	CRT-p reduced all-cause mortality, hospitalizations and improved NYHA, quality of life
REVERSE (Linde et al, 2008)	610	Double-blinded, randomized – CRT-ON *vs* CRT-OFF, 12 months	NYHA I–II, left ventricular ejection fraction <40%, QRS >120 msec	Primary – % worsened heart failure clinical composite, secondary – left ventricular end systolic volume index, heart failure hospitalizations, all-cause mortality	CRT-p/CRT-d did not change the primary endpoint, reduced left ventricular end systolic volume index, heart failure hospitalizations
MADIT-CRT (Moss et al, 2009)	1820	Single-blinded, randomized – CRT-d *vs* internal cardiac defibrillator, 12 months	NYHA I–II, left ventricular ejection fraction <30%, QRS >130 msec	Primary – all-cause mortality or heart failure hospitalizations, secondary – all-cause mortality, left ventricular end systolic volume	CRT-d reduced the primary endpoint and left ventricular end systolic volume, CRT-d did not reduce all-cause mortality
RAFT (Tang et al, 2010)	1798	Double-blinded, randomized – CRT-d *vs* internal cardiac defibrillator, 40 months	NYHA II–III, left ventricular ejection fraction <30%, QRS >120 msec	Primary – all-cause mortality or heart failure hospitalizations, secondary – all-cause mortality and cardiovascular death	CRT-d reduced primary endpoint CRT-d (NYHA III) reduced all-cause mortality

CRT-d = cardiac resynchronization therapy-defibrillator; CRT-OFF = cardiac resynchronization therapy – turned OFF; CRT-ON = cardiac resynchronization therapy – turned ON; CRT-p = cardiac resynchronization therapy-pacemaker; NYHA = New York Heart Association. Adapted from Brignole et al (2013).

and sinus rhythm.

Unfortunately, not all patients who have heart failure benefit from cardiac resynchronization therapy. Multiple randomized controlled trials have informed and refined implantation criteria of national and international guidelines. Benefit has consistently been demonstrated in patients with severe left ventricular systolic dysfunction (left ventricular ejection fraction ≤35%). Those with moderate impairment (left ventricular ejection fraction 35–45%) can be offered cardiac resynchronization therapy if they have a bradycardia pacing indication and are likely to require >40% pacing (Curtis et al, 2013).

New York Heart Association classification

NYHA symptom classification as a criterion initially favoured more symptomatic patients in class III or IV (*Table 1*). Interestingly, these trials consistently recruited a substantially lower proportion of patients with NYHA IV symptoms (representing 7–15%) (Brignole et al, 2013). More recent randomized controlled trials have included patients with milder heart failure symptoms (NYHA I–II) and demonstrated improvement in cardiovascular outcomes and reverse left ventricular remodelling. However, NYHA class I patients represented a small proportion of the participants in all trials and no benefit was specifically seen for these in sub-group analyses (Linde et al, 2008; Moss et al, 2009).

QRS duration

QRS duration is one of the most powerful predictors of benefit from cardiac resynchronization therapy. Sub-group analyses of MADIT-CRT (Moss et al, 2009; Hsu et al, 2012), REVERSE (Linde et al, 2008) and RAFT (Tang et al, 2010) consistently demonstrated that patients with QRS durations ≥150 msec on resting 12-lead electrocardiogram have the greatest reduction in cardiovascular outcomes.

Cleland et al (2013) performed a large meta-analysis (n=3782) from five Medtronic Ltd (Minneapolis, USA) sponsored randomized controlled trials comparing cardiac resynchronization therapy with no active treatment or cardiac resynchronization therapy-defibrillator with implantable cardiac defibrillators. Several pre-defined variables were evaluated to identify if they predicted a composite outcome of all-cause mortality and/or first heart failure hospitalization (Cleland et al, 2013). Patients with atrial fibrillation and NYHA I symptoms were excluded from analysis as they only comprised a small proportion of patients. Cleland et al (2013) accounted for the influence of having cardiac resynchronization therapy and treated it as a fixed effect variable in the prediction models. Incremental increase in QRS duration on pre-implant electrocardiogram showed a magnitude of benefit for improving cardiovascular outcomes after cardiac resynchronization therapy implant for every additional millisecond. Definitive benefit was observed from 140 msec onwards and plateaued beyond 180 msec for composite outcome alone (Cleland et al, 2013). This meta-analysis has been an important milestone in reviewing the evidence behind the recommendations on QRS durations.

Patients with narrower QRS duration (120–130 msec) do not benefit from cardiac resynchronization therapy implantation (Cleland et al, 2013). The Cardiac Resynchronization Therapy in Heart Failure with a Narrow QRS Complex (EchoCRT) trial (Ruschitzka et al, 2013) enrolled patients (n=855) across 115 centres. These patients met standard implantation criteria having a QRS complex ≤130 msec with evidence of cardiac dyssynchrony on echo to CRT-ON (cardiac resynchronization therapy capability turned on) or CRT-OFF (cardiac resynchronization therapy capability turned off) following implantation. The primary outcome was a clinical composite of all-cause mortality and heart failure hospitalization. The EchoCRT trial demonstrated CRT-ON had a higher rate of composite primary end-point occurrence compared with CRT-OFF (28.7% *vs* 25.2%, P=0.15). However, all-cause mortality was significantly higher in the CRT-ON group compared with CRT-OFF (11.1% *vs* 6.4%, P=0.02) (Ruschitzka et al, 2013). The greatest benefit of cardiac resynchronization therapy was seen in those with the widest QRS duration.

QRS morphology: bundle–branch block

QRS morphology is important in determining response to cardiac resynchronization therapy. Sub-group analyses of MADIT-CRT (Moss et al, 2009), RAFT (Tang et al, 2010) and REVERSE (Linde et al, 2008) trials all identified complete left bundle–branch block as having better outcome on all-cause mortality and hospitalization compared with right bundle–branch block and non-specific intraventricular conduction delay. A meta-analysis by Cunnington et al (2015), including 6914 patients, analysed those with and without left bundle–branch block. Participants across all included trials had NYHA I–IV symptoms, left ventricular ejection fraction ≤30–40% and QRS duration ≥120–130 msec. The study demonstrated no benefit from cardiac resynchronization therapy for patients with non-left bundle–branch block QRS morphology for pooled outcome of all-cause mortality and heart failure hospitalization (hazard ratio 1.09, 95% confidence interval 0.85–1.39). It should be noted that Cunnington et al (2015) only studied cardiovascular end-points and did not examine symptom, functional or echocardiographic outcomes. It was also acknowledged that NYHA classes I and IV were under represented and observations were driven by those with NYHA class II–III symptoms.

The MADIT-CRT trial (Moss et al, 2009) of NYHA I–II patients (n=536), followed over 7 years, demonstrated increased risk of mortality for non-left bundle–branch block patients (hazard ratio 1.57, 95% confidence interval 1.03–2.39).

Separating QRS duration from bundle–branch block morphology remains a challenge for cardiac resynchronization therapy. Both variables are important for selecting suitable candidates. Different bundle–branch block patterns have been demonstrated on activation

mapping studies to have heterogeneous patterns and should be considered as different entities (Varma, 2009). In the meta-analysis by Cleland et al (2013) left bundle–branch block was associated with broader QRS durations, suggesting the power of increasing QRS duration to infer better cardiovascular outcomes was confounded by bundle–branch block morphology; it also observed that non-left bundle–branch block had an increased trend towards higher mortality. However, when QRS duration was removed from the multivariable prediction model, little difference was noted between left bundle–branch block and non-left bundle–branch block in terms of impact on mortality (Cleland et al, 2013). Together these observations suggest that bundle–branch block and QRS duration are intertwined variables, which may need to be considered together when reviewing a patient for cardiac resynchronization therapy.

Atrial fibrillation

Atrial fibrillation commonly co-exists in patients with heart failure and its presence can reduce the success of cardiac resynchronization therapy (Wilton et al, 2011). Understanding the true influence of atrial fibrillation on the success of cardiac resynchronization therapy is difficult as patients with atrial fibrillation tend to be older, have more comorbidities and be more unwell. Comparison between sinus rhythm and atrial fibrillation is influenced by these confounding factors, which often infer worse prognosis (Brignole et al, 2013). Atrial fibrillation is underrepresented in randomized controlled trials of cardiac resynchronization therapy and so meta-analysis is needed. Patients with atrial fibrillation receiving cardiac resynchronization therapy have a similar improvement in left ventricular ejection fraction to those in sinus rhythm, but have worse symptom and functional response (Wilton et al, 2011). In a large (n=7495) meta-analysis of 33 observational studies, Wilton et al (2011) compared those with atrial fibrillation (22.5%) to those with sinus rhythm receiving cardiac resynchronization therapy and observed a significantly higher all-cause mortality and non-responder rate in the atrial fibrillation group. Evidence on the precise recommendations for cardiac resynchronization therapy in patients with atrial fibrillation remains weak and is based on limited evidence and expert opinion. However, implantation is favoured if >99% biventricular pacing percentage can be achieved (Brignole et al, 2013).

Cardiac resynchronization therapy implantation criteria

The current implantation criteria have been significantly modified over the last 15 years as more evidence has been produced and collated. The previous sections have discussed the evolution and refinement of the current evidence, which reflects the current international guidelines (Daubert et al, 2012). These have changed to incorporate the most recent evidence, including patients with atrial fibrillation and bradycardia pacemaker indications (Brignole et al, 2013). In June 2014 in the UK, the National Institute for Health and Clinical Excellence revised guidance on cardiac resynchronization therapy implantation that reflected updated international guidelines (National Institute for Health and Clinical Excellence, 2014) (*Table 2*). Current indications are heart failure with reduced ejection fraction (left ventricular ejection fraction ≤35%) with NYHA II–IV symptoms on optimal medical therapy and with QRS duration on resting electrocardiogram of either: 120–149 msec with left bundle–branch block morphology or ≥150 msec duration (this includes NYHA class I patients). Patients in atrial fibrillation who can be rate controlled (by medication or atrioventricular node ablation) and fulfil cardiac resynchronization therapy implant criteria can be considered too. Patients with impaired left ventricular function who are anticipated to require ventricular pacing >40% of the time should also be considered for cardiac resynchronization therapy (Brignole et al, 2013).

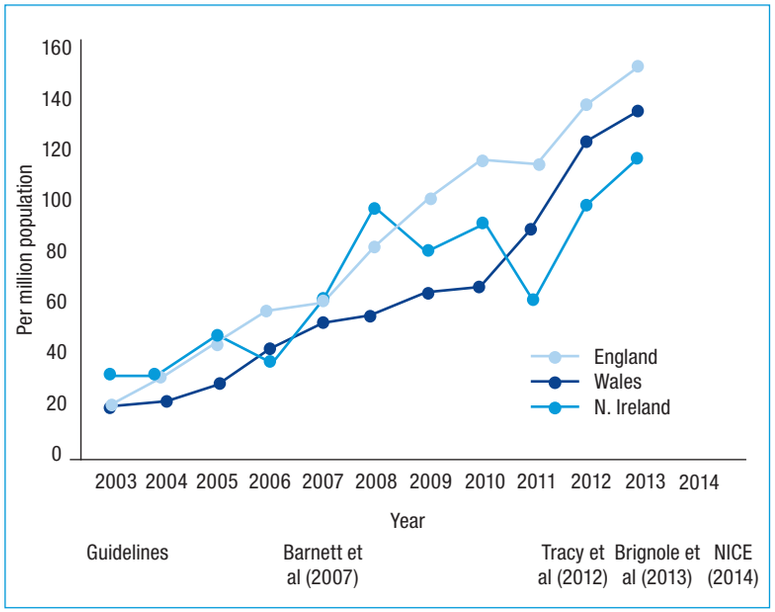
The European Society of Cardiology introduced new recommendations for cardiac resynchronization therapy implantation in August 2016 (Ponikowski et al, 2016).

Table 2. National Institute for Health and Clinical Excellence indications for implantable cardiac defibrillator and cardiac resynchronization therapy in patients with left ventricular ejection fraction ≤35%

	NYHA class			
QRS interval	**I**	**II**	**III**	**IV**
<120 msec	Internal cardiac defibrillator if there is a high risk of sudden cardiac death		Internal cardiac defibrillator and cardiac resynchronization therapy not clinically indicated	
120–149 msec without left bundle–branch block	Internal cardiac defibrillator	Internal cardiac defibrillator	Internal cardiac defibrillator	CRT-p
120–149 msec with left bundle–branch block	Internal cardiac defibrillator	CRT-d	CRT-p or CRT-d	CRT-p
>150 msec	CRT-d	CRT-d	CRT-p or CRT-d	CRT-p

Adapted from National Institute for Health and Clinical Excellence (2014). CRT-d = cardiac resynchronization therapy-defibrillator; CRT-p = cardiac resynchronization therapy-pacemaker; New York Heart Association.

Figure 2. Total cardiac resynchronization therapy implant rates 2003–13 and release of national and international implantation criteria. Adapted from Cunningham et al (2014). NICE = National Institute for Health and Clinical Excellence.



Referring to the issues raised by the strength of evidence about implanting cardiac resynchronization therapy into patients with a low QRS 120–130 msec raised by Cleland et al (2013) and the ECHO CRT study (Ruschitzka et al, 2013), these guidelines recommended that cardiac resynchronization therapy should now be implanted in patients with a QRS ≥130 msec. The National Institute for Health and Clinical Excellence (2014) guidance still remains as in *Table 2*, but will possibly change when guidelines are updated in August 2018.

Cardiac resynchronization therapy implantation in the UK

Over the last decade several hundred thousand cardiac resynchronization devices have been implanted worldwide (Daubert et al, 2012). In 2013 the UK was the fourth highest total cardiac resynchronization therapy implanter within western Europe (Cunningham et al, 2014). *Figure 2* demonstrates the increasing year-on-year implantation rate within the home nations of the UK, over the last decade. Figures for Scotland are not presented because the data are incomplete (Cunningham et al, 2014). These figures demonstrate the establishment of cardiac resynchronization therapy as a cornerstone of heart failure management. Implantation rates continue to increase with broadening of implantation guidelines and more centres starting to implant.

Health economics of cardiac resynchronization therapy

Despite recent revision of international guidelines and more focused studies on cardiac resynchronization therapy response, evidence suggests a non-response rate of 20–30% (Bristow et al, 2004; Cleland et al, 2005; Moss et al, 2009). There is a strong focus on trying to predict and minimize this non-response rate. Cardiac resynchronization therapy implantation is a costly intervention with a large up-front cost of an estimated £3411 for a cardiac resynchronization therapy-pacemaker and £12 293 for a cardiac resynchronization therapy-defibrillator (National Institute for Health and Clinical Excellence, 2014). The up-front cost is larger than for many other medical devices, and there are also ongoing costs of monitoring and replacing these devices (Boriani et al, 2009).

Randomized controlled trials have been used to model the quality-adjusted life year costs of a cardiac resynchronization therapy device. It is widely accepted that this falls below $50 000 per quality-adjusted life year, which is the accepted cost of an intervention in the USA (Boriani et al, 2009). Efforts have focused on minimizing this cost by better defining the heart failure population who will benefit, streamlining implantation and using remote monitoring to reduce patient visits to hospital (Boriani et al, 2009). However, the burden of cost to health-care systems will continue to rise with the growing population of patients with heart failure who might benefit from cardiac resynchronization therapy. More accurately defining the non-response rate will minimize this burden of cost.

The challenge of non-response

Despite two decades of large randomized controlled trials, detailed meta-analyses and observational studies, there remains an unchanging minority (20–30%) of heart failure patients meeting cardiac resynchronization therapy implant criteria who fail to respond (Bristow et al, 2004; Cleland et al, 2005; Moss et al, 2009). QRS duration and morphology have consistently been shown to be the strongest predictors of cardiovascular outcomes. Cleland et al (2013) clearly demonstrated the pooled magnitude of strength of ever-increasing QRS duration beyond >140 msec to predict benefit. QRS morphology does not demonstrate such clear strength to predict response (Cleland et al, 2013), although it is clear that non-left bundle–branch block morphology favours poorer response (Cunnington et al, 2015).

Questions still remain around the benefit of cardiac resynchronization therapy in patients with QRS durations 120–140 msec and the additional benefit that left bundle–branch block morphology offers (Cunnington et al, 2015). Calls have been made for randomized control trials of patients with cardiac resynchronization therapy already in-situ, who have narrow native QRS with non-left bundle–branch block morphology on electrocardiogram, to have their devices deactivated for a period of observation, given the question over benefit (Cleland and Freemantle, 2015). Moreover the apparent influence of bundle–branch block morphology on QRS duration >150 msec remains unclear.

Beyond current indications for cardiac resynchronization therapy and QRS durations and morphology many other predictors have been identified. Sub-studies of the large cardiac resynchronization therapy randomized controlled

trials have identified multiple variables that improve morbidity and mortality. In a sub-study of the MADIT-CRT trial, Hsu et al (2012) performed a best-subset regression on patients who had paired echocardiograms at 12 months and had been assigned to have cardiac resynchronization therapy-defibrillator (n=752) to examine for predictors of echo super-responders (top quartile of left ventricular ejection fraction change). Six predictors were identified as being associated with left ventricular ejection fraction super-response: female gender (odds ratio 1.96, P=0.001), no prior myocardial infarction (odds ratio 1.80; P<0.01), QRS duration ≥150 msec (odds ratio 1.79, P<0.01), left bundle–branch block (odds ratio 2.05, P<0.01), body mass index <30 kg/m^2 (odds ratio 1.51, P=0.035), and smaller baseline left atrial volume index (odds ratio 1.47, P=0.001). The impact of cardiac resynchronization therapy was not accounted for in this analysis (Hsu et al, 2012); furthermore this sub-study required paired echocardiograms, thereby favouring patients who had survived 12 months, creating a selection bias.

Cleland et al (2013) examined multiple predefined potential predictor variables (age, gender, NYHA class, heart failure aetiology, QRS morphology, QRS duration, left ventricular ejection fraction and systolic blood pressure) and only QRS duration could predict cardiac resynchronization therapy outcomes. Cleland and Freemantle (2015) consistently argued that much of the evidence for predictors is based upon sub-group analyses. Moreover, in these subset studies and meta-analyses based upon the cardiac resynchronization therapy randomized controlled trials the impact of the device was not accounted for and confounded the results observed.

Many observational studies have been performed to examine the potential of different variables to predict cardiac resynchronization therapy response and outcome. These observational studies tend to be of limited value, often being under-powered, having flawed methodology, not accounting for cardiac resynchronization therapy implantation and using a variety of different response and outcome definitions. Their value shadows that of the often quoted cardiac resynchronization therapy randomized controlled trial sub-studies and more importantly meta-analyses. However, observational studies tend to emphasize the value of response in terms of patient-centred criteria (symptoms, function and quality of life) compared with the composite cardiovascular outcomes of most cardiac resynchronization therapy trials (*Table 1*). Well-conducted observational studies often generate new lines of hypothesis and investigation, so they still have value in the investigation of non-response. *Table 3* summarizes important observational studies evaluating clinical predictors.

Responder definition

The consistent issue examining cardiac resynchronization therapy response is the variety of different definitions used in the literature. This makes comparing and pooling data for comparison difficult because of the heterogeneity of

Table 3. Observational studies assessing predictors of non-response

Study	Patients	Study design	Inclusion	Response criteria	Main findings
Shanks et al (2011)	581	Observational study, single centre (Holland), 6 months	Not clear which NYHA, left ventricular ejection fraction ≤35%, QRS ≥120 msec	Clinical and echocardiographic: ↓NYHA ≥1 and survival and no heart transplantation ↑>15% left ventricular end systolic volume	Predict non-response: ischaemic aetiology, shorter 6-minute walk distance at baseline, less baseline cardiac dyssynchrony and left ventricle lead position
Lin et al (2014)	193	Retrospective observational study (China), single centre, all consecutive cardiac resynchronization devices, 12 months	NYHA II–IV, left ventricular ejection fraction ≤35%, QRS ≥120 msec	Echocardiographic: ↑≥5% left ventricular ejection fraction and survived and being free from heart failure hospitalization	Predicts non-response: non-left bundle–branch block and non-optimal left ventricle lead position
Rinkuniene et al (2014)	82	Retrospective observational study, single centre (Lithuania), 12 months	NYHA III–IV, left ventricular ejection fraction ≤35%, QRS ≥120 msec, left bundle–branch block	Clinical: ↓≥1 NYHA and ↑>15% 6-minute walk distance echocardiographic: ↑≥15% left ventricular end systolic volume	Predicts response: non-ischaemic aetiology (clinical) and left ventricular end diastolic diameter (≤75 mm) (echo)
Sassone et al (2015)	243	Retrospective observational study, all consecutive cardiac resynchronization devices, majority cardiac resynchronization therapy-defibrillator, single centre (Italy), 6 months, left bundle–branch block in predictor analysis	NYHA II–IV, left ventricular ejection fraction <35%, QRS >120 msec	Echocardiographic: ↑>15% left ventricular end systolic volume. Clinical composite: heart failure hospitalization, mortality and first sustained ventricular tachycardia	Predict non-response: ischaemic aetiology and QRS duration (≥178 msec). Clinical composite: non-left bundle–branch block ↑ rate events

NYHA = New York Heart Association.

KEY POINTS

- Cardiac resynchronization therapy should be considered in all patients with severe left ventricular systolic dysfunction with evidence of electrical dyssynchrony on resting 12-lead electrocardiogram refractory to optimal medical therapy.
- Cardiac resynchronization therapy significantly reduces mortality and hospitalizations in heart failure patients with broad QRS.
- Cardiac resynchronization therapy resynchronizes both atrioventricular and inter-/intra-ventricular dyssynchrony thereby promoting reverse cardiac remodelling, which improves left ventricular ejection fraction and reduces left ventricular end systolic volume and functional mitral regurgitation.
- The benefit of cardiac resynchronization therapy increases incrementally with increasing QRS duration (with benefit beginning at QRS duration >130 msec).
- Patients with at least moderate left ventricular impairment and bradycardia pacing indication and likely to require >40% ventricular pacing should be offered cardiac resynchronization therapy.
- A significant challenge of cardiac resynchronization therapy remains the 20–30% non-response rate.

criteria used for response. Fonwalt et al (2010) performed a seminal systematic review of the 26 most cited papers on predicting cardiac resynchronization therapy response and extrapolated 17 different criteria. Fifteen criteria (clinical and echocardiographic) were all applied to the PROSPECT trial cohort (two criteria could not be calculated). The application of these different definitions to the same cohort demonstrated a response rate variation between 32% and 91% (Chung et al, 2008; Fornwalt et al, 2010). Agreement was poor (105 combinations) between 79 (75.2%) pairs of definitions (Fornwalt et al, 2010). Moreover, a strong association of agreement was only observed in four (3.8%) pairs of definitions. All echocardiographic and clinical definition combinations had a poor association. Removal of definitions applied in short-term follow up (<3 months) made no significant change to the analysis. Agreement between definitions is poor, even between similar categories of criteria (Fornwalt et al, 2010).

In a paper on heart failure composite scores, Packer (2001) identified the pitfalls of using one individual metric to measure response; composite scores can minimize this problem. No universal response definition has yet been agreed upon, but the consensus is that a combination of composite criteria is required. Criteria should not combine clinical or echocardiographic variables (Fornwalt et al, 2010).

Conclusions

Cardiac resynchronization therapy has revolutionized the treatment and outcomes for selected heart failure patients who are refractory to optimal medical therapy and exhibit evidence of dyssynchrony on resting 12-lead electrocardiogram. In the correct patient, cardiac resynchronization therapy reverses adverse cardiac remodelling; the process that underpins the development and progression of heart failure with reduced ejection fraction. Refinement of evidence over the last 15 years has led to changes in national and international guidelines for cardiac resynchronization therapy. QRS duration on electrocardiogram remains the most important factor determining response to cardiac resynchronization therapy. Emerging evidence suggests that QRS durations 120–130 msec do not confer any benefit. Unfortunately, despite refinements in implant criteria, a significant cardiac resynchronization therapy non-response remains (20–30%). Research continues on being able to better identify and stratify patients before cardiac resynchronization device implantation. Cardiac resynchronization therapy remains one of the greatest advances in the last 20 years for the management of heart failure with reduced ejection fraction. All patients who have a significant reduction in left ventricular ejection fraction and have broad QRS duration on resting electrocardiogram should be considered for implantation of cardiac resynchronization therapy.

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Extracellular cardiac matrix biomarkers in patients with reduced ejection fraction heart failure as predictors of response to cardiac resynchronisation therapy: a systematic review

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ABSTRACT

Objective Cardiac resynchronisation therapy (CRT) is an effective therapy for selected patients with heart failure (HF); however, a significant non-response rate exists. We examined current evidence on extracellular cardiac matrix (ECM) biomarkers in predicting response following CRT.

Methods Complete literature review of PubMed, Ovid SP MEDLINE, Cochrane Library and TRIP, reference lists, international cardiology conferences and ongoing studies between December 1999 and December 2015 conducted according to prospectively registered study selection and analysis criteria (PROSPERO:CRD42016025864) was performed. All observational and randomised control trials (RCT) were included if they tested prespecified ECM biomarkers' ability to predict CRT response. Risk of bias assessment and data extraction determined pooling of included studies was not feasible due to heterogeneity of the selected studies.

Results A total of 217 studies were screened; six (five prospective cohort and one RCT substudy) were included in analysis with 415 participants in total. Study sizes varied (n=55–260), cohort characteristics contrasted (male: 67.8%–83.6%, ischaemic aetiology: 40.2%–70.3%) and CRT response definitions differed (three clinical/functional, three echocardiographic). Consistent observation in all ECM biomarker behaviour before and after CRT implantation was not observed between studies. Lower type I and type III collagen synthesis biomarkers (N-terminal propeptides of type I and III procollagens) expression demonstrated replicated ability to predict reverse left ventricular remodelling.

Conclusion Collagen synthesis biomarkers offer the most potential as ECM biomarkers for predicting CRT response. Heterogeneity between these studies was large and limited the ability to pool and compare results numerically. Use of different response definitions was one of the biggest challenges.

KEY QUESTIONS

What is already known about this subject?

- Cardiac resynchronisation therapy (CRT) is associated with non-response in 20%–40% of selected patients with heart failure (HF). Selected vascular biomarkers are known to be associated with cardiac disease but it is unknown whether these can be used to predict CRT response.

What does this study adds?

- We performed a systematic review of all studies examining vascular biomarkers in CRT. We found that collagen synthesis biomarkers have the most potential for predicting CRT response, particularly N-terminal propeptides of type I and III procollagens. Matrix metalloproteinases-2 and 9 have no conclusive predictive value and need further investigation.

How might this impact clinical practice?

- Use of vascular biomarkers to predict CRT response could have enormous clinical benefit by selectively identifying those patients with HF who are likely to benefit. This has important implications for both patients and healthcare providers worldwide, especially given the current financial climate.

INTRODUCTION

Cardiac resynchronisation therapy (CRT) is an effective therapy for selected patients with heart failure (HF).[1 2] Current guidelines suggest that CRT is offered to those with a left ventricular ejection fraction (LVEF) ≤35% with resting 12-lead ECG QRS duration ≥150 ms or 120–149 ms with Left Bundle Branch Block (LBBB) morphology and refractory to optimal medical therapy (OMT).[3] CRT reduces mortality and improves morbidity, underpinned by reversal of pathophysiological adverse cardiac remodelling.[1 2] Unfortunately, a significant non-response rate of 20%–40% exists and has remained unchanged over the last decade, despite extensive research and investment.[1 2]

The extracellular cardiac matrix (ECM) is a dynamic support structure that remodels following cardiac injury and HF.[4 5] Progressive ECM remodelling is closely linked to HF severity and prognosis.[4 5] Cardiac collagen turnover alterations are central to the development and progression of cardiac fibrosis and HF.[5] Specific biomarkers of type I and type III collagen synthesis (N-terminal propeptides of type I and III procollagens (PINP and PIIINP),[6 7] carboxy-terminal propeptide of procollagen type I (PICP))[8 9] and degradation (carboxy-terminal telopeptide of type I collagen (ICTP or CITP))[9 10] products are associated with poor outcomes in HF. The proteolytic enzyme system matrix metalloproteinases (MMPs) and their regulators tissue inhibitors of MMPs (TIMPs) are involved in collagen degradation and have been implicated in HF development and progression.[4 5] Specifically, MMP-1,[11] a collagenase, MMP-2[12] and MMP-9,[13] both gelatinases and TIMP-1[11] are associated with HF outcomes. Galectin-3 (Gal-3) is a beta-galactoside-binding lectin released by activated cardiac macrophages, which are upregulated in HF, causing increased fibroblast proliferation, collagen deposition and ventricular dysfunction.[14] Gal-3 is strongly associated with inflammation and fibrosis with raised levels strongly predict poor HF outcomes.[14]

Turnover of ECM alters in HF and with reverse cardiac remodelling following CRT implantation may offer potential biomarkers for response prediction.[15] This systematic review examines the current evidence on the value of ECM biomarkers in predicting CRT response.

METHODOLOGY

Our systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines.[16] It was prospectively registered with PROSPERO (CRD42016025864), an international registry of systematic reviews. A protocol was designed and implemented prospectively in-line with PRISMA-P 2015.[17]

Eligibility criteria

Strict eligibility criteria were applied to minimise heterogeneity of included articles. Observational studies (prospective or retrospective) and randomised control trials (RCTs) (including substudies) were included; basic science and review articles were excluded. Included study populations represented patients with HF meeting international CRT implant guidelines.[3] Studies had to be conducted on adults (age ≥18 years). Articles were included if they examined an ECM biomarker previously reported to predict HF outcomes.[4] Baseline ECM biomarkers, measured when patients were clinically stable prior to implantation, had to be compared with a predefined CRT 'response' criteria to evaluate their predictive value. Coronary sinus sampling and long-term trends in peripheral ECM biomarker behaviour were analysed if present.

A variety of clinical, functional or echocardiographic criteria and cardiovascular outcomes have been used to define CRT response in studies,[18] which often correlate poorly. All response criteria were included in the review. Cardiovascular outcomes could form part of a response definition or be presented separately; their absence was not an exclusion criterion.

Database search strategies

Detailed searches were conducted on PubMed, Ovid SP MEDLINE, Cochrane Library (CENTRAL) and TRIP in February 2016 by one author (CM) and reviewed by another independently (DA). The search strategy used specific terms (cardiac resynchronisation therapy/cardiac pacing/extracellular matrix) in combination, within titles/abstracts or Medical Subject Headings. Specific vascular biomarkers ('TIMP' 'MMP' 'collagen' 'Myostatin' 'Syndecan-4' and 'Galectin-3') were included in the search. A grey literature search involved searching the Clinical Trials database (www.clinicaltrials.gov) and international cardiology conferences (European Society of Cardiology, American Heart Association, American College of Cardiology) indexes for ongoing, abstracts and unpublished work. All included articles had their references searched for relevant publications. A date limitation of the last 15 years (31 December 1999–31 December 2015) was applied. No language restrictions were applied.

Title and abstract reviews were performed independently (CM/DA), consensus on eligibility criteria was required to be taken forward to full paper review; any conflicts were decided by an independent reviewer (FO). Duplications of articles or cohort use were identified and only the most relevant (decided by consensus) taken forward. The Critical Appraisal Skills Programme checklist (dependent on study design) was applied to full paper review to guide evaluation of article quality.[19] Consensus had to be reached on full paper reviews before being selected for inclusion; where consensus was not reached a third reviewer (FO) made the final decision. Contact was attempted with all included article authors and any others at full paper review that were indicated.

Data extraction and management

Full texts of included articles were obtained. Pilot data extraction was performed on two randomly selected articles and reviewed for robustness (CM, DA, FO, PB). A standardised data extraction form was created to collect data on each study's design (eligibility criteria, methodology, assessment period), patient population (numbers, age, gender, aetiology, ECG, left ventricular (LV) geometry, quality of life, New York Heart Association (NYHA), functional assessment), vascular biomarker/predictor (specific ECM surrogate biomarkers, units, conditions of sampling, laboratory assessment, statistical prediction model) and outcome (response definition and cardiovascular outcomes). Data extraction was performed by two independent

reviewers (CM/DA), a third independent reviewer (FO) resolved any disagreement.

Risk of bias assessment

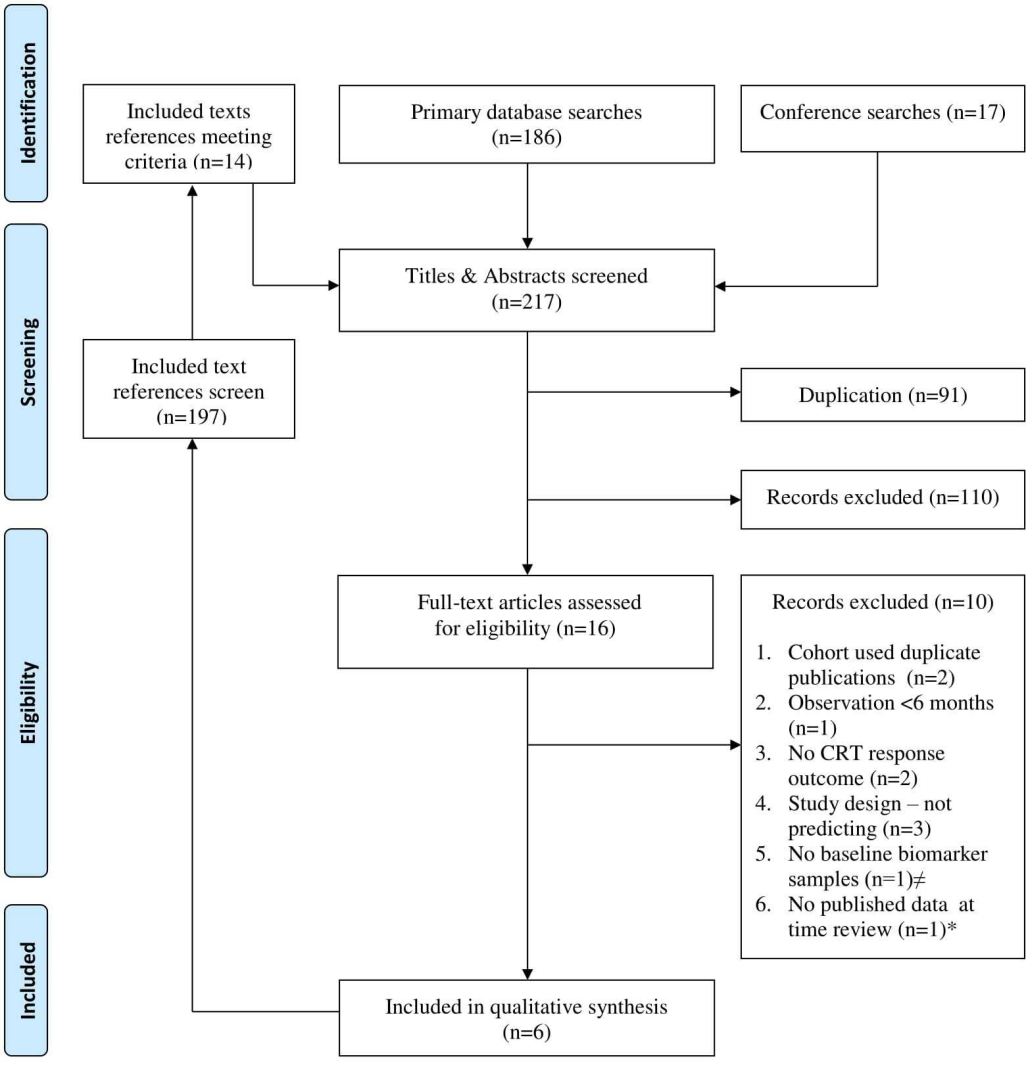
Risk of bias for each study was assessed by two independent reviewers (CM, DA) utilising either the Risk of Bias Assessment Tool for Non-randomised Studies or the Cochrane Collaboration 'Risk of Bias' assessment tool.[20 21] Both have established criteria to examine selection bias, exposure measurement, blinding and completeness of outcome data.[20 21]

Data synthesis and analysis

A descriptive synthesis was performed to summarise findings of all selected articles. A meta-analysis of included study data for each specific ECM biomarker was not possible due to heterogeneity of outcome definitions and study designs. Evaluation of study designs, defined outcomes and cohort characteristics was performed. The same biomarkers compared in different included articles were compared. Continuous variables were summarised using the same units for each variable in the original text. Data were presented as mean ± standard deviation (SD), unless specified otherwise.

RESULTS

Figure 1 shows the screening and selection of published articles; 110 records were excluded after the screening stage as they did not meet inclusion criteria. Six articles met the inclusion criteria. Two abstracts[22 23] and one clinical trial entry (www.clinicaltrials.gov) (NCT15019908) were



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For more information, visit www.prisma-statement.org.

Figure 1 Flowchart of studies screening and selection. ≠Author contacted, poster presentation sent and no baseline extracellular cardiac matrix biomarker sample taken.[22] *Clinical trial (NCT15019908) author contacted and manuscript in preparation. CRT, cardiac resynchronisation therapy.

taken to full review (for potential inclusion). Related articles and information were sought, including contacting authors (all three kindly responded). None yet had articles published and additional information provided led to exclusion from review (no baseline biomarkers taken[22] or study design did not test biomarkers as predictors[23]).

Study design

Five prospective cohort studies and one RCT substudy[11] were included. Table 1 summarise the different study designs and CRT response outcome definitions used. Studies selected were published between 2008 and 2014. Risk of bias was assessed in each study using appropriate quality check tools. The lowest risk of bias was in the single RCT substudy.[11] The prospective cohort studies varied minimally in their bias assessment and none were excluded.

Garcia-Bolao *et al*[9] stated that 61 participants were consented; during the observation period there were four mortalities (three cardiac/one non-cardiac) and one functional assessment not performed at follow-up (6 min walk test not completed due to stroke). The cohort was 59 but no explicit statement about the two exclusions made. Lopez-Andres *et al*[11] published a substudy in 2012 of the 'The Effect of Cardiac Resynchronization on Morbidity and Mortality in Heart Failure' (CARE-HF)[1] RCT which itself was published in 2005; interpretation of results is within this context. All studies included NYHA III–IV patients (mostly NYHA III). Two studies recruited NYHA II patients[24 25] with one also requiring a bradycardia pacing indication.[24] All studies included QRS duration >120 ms, except Garcia-Bolao *et al*[9] (QRS≥130 ms). In the CARE-HF trial, those with QRS duration 120–149 ms needed dyssynchrony on echocardiography.[1 8] All transvenous LV leads were implanted preferably to the most lateral position possible. Dong *et al*[26] performed only de novo CRT-defibrillator (CRT-d) implants. Three studies[10 24 26] commented on right ventricular lead placement with two[26] explicitly aiming for the right ventricular apex. In CARE-HF (and substudy), all had CRT-pacemaker (CRT-p) devices only.[1 11] CRT response definitions varied between included studies. Broadly, response definitions used were classified as three clinical and three echocardiographic. Reported response rates varied between 48.9% and 71.8% (table 1).

Baseline characteristics

The baseline characteristics of the patients are shown in table 2.

A total of 415 patients were included. The five prospective observational studies had mean age of 67±10 years[9 10] (Lopez-Andres *et al*[8] excluded as presented as median and IQR). There were 315 (75.9%) males in included studies, ranging 67.8%[9]–83.6%.[25] There was large variation in frequency of CRT-d/CRT-p implants in each study with two not providing this data.[10 25] One study included a high proportion of device upgrades[25]; the CARE-HF trial excluded upgrades,[1 8] the remaining four studies did not state upgrade status.[9 10 24 26] Atrial fibrillation (AF) was included in three prospective observational studies[8 24 25]; one did not report on AF or related publications.[10 27] Precise QRS duration was not stated in two studies.[24 26] Reporting of LV volumetric data varied between included studies. Three reported unadjusted LV end systolic volume (LVESV) and LV end diastolic volume (LVEDV) data which were similar to each other (table 3).[10 24 25] Dong *et al*[26] presented LVESV and LVEDV volume indexed figures only. Garcia-Bolao *et al*[9] provided LVEF only. LVEF was compared between the five prospective cohorts and showed similar mean LVEF between 25%–27%.[9 10 24–26]

Responder versus non-responders

Response status (responders vs non-responders (RvsNR)) was presented in four of the included studies.[9 10 24 26] Truong *et al*[25] did not provide characteristics of those defined by response. Lopez-Andres *et al*[8] outlined characteristics by allocation to CRT-p versus OMT, however, not by response. There were some baseline characteristic differences between the four studies for RvsNR[9 10 24 26]; Dong *et al*[26] demonstrated differences between RvsNR for LBBB status (15 (68.3%) vs 9 (39.1%), p=0.05) and ischaemic aetiology (9 (40.9%) vs 17 (73.9%], p=0.03). Tolosana *et al*[24] reported lower creatinine levels in RvsNR (1.25±0.3 mg/dL vs 1.76±0.8 mg/dL, p=0.01). Umar *et al*[10] reported that responders were older and had longer QRS duration than non-responders (age: 66±10 years vs 60±11 years, p=0.03; mean± standarderror QRS: 165±3 ms vs 135±8 ms, p=0.001). Notably, Hessel *et al* published a study using the same cohort as Umar *et al* and reported no difference in QRS duration for RvsNR (165±2 ms vs 153±3 ms, p=NS), suggesting one of these studies has recorded it incorrectly.[10 27]

ECM biomarkers

All ECM biomarker baseline concentrations and magnitude of association (if tested) are summarised in table 3. Lopez-Andres *et al*[8] did not provide baseline concentrations by response status, but comparison was made with the control group. Umar *et al*[10] showed baseline results for expression of ECM biomarkers studied. However, for PIIINP non-responders no baseline concentration was reported in the article, however no statistical significance is reported RvsNR.[10]

PINP/PICP

PINP and PICP share a 1:1 stoichiometric relationship with the collagen molecule; therefore, they were considered together. Umar *et al*[10] reported similar total cohort means values to Lopez-Andres *et al*[8] median values (the skew of this data is unknown). Umar *et al*[10] observed higher PINP baseline level predicted poor response. Garcia-Balao *et al*[9] reported the opposite for PICP. Lopez-Andres *et al*[8] observed no significant association of baseline PINP with CRT response or other outcomes. Variation in the pattern of reported levels between the

Table 1 Study designs and response outcome definitions

Study ID	Design	Participants recruited	HF/CRT participants	Inclusion criteria	Observation period	Assessments	ECM biomarkers	CRT responder definition	Response rate
Dong *et al*[26]	Prospective observational	65 (20 healthy controls)	45	LVEF≤35%, NYHA III–IV, QRS>120 ms, SR and OMT	6 months	Baseline/3 months/6 months: NYHA, 6MWT, TTE, blood samples	PIIINP	↓≥15% LVESVi and survived at 6 months	22 (48.9%)
Tolosana *et al*[24]	Prospective observational	55 (13 excluded after recruitment)	42	LVEF≤35%, NYHA III–IV, QRS>120 ms and OMT or cardiac pacing indication (LVEF<35%)	12 months	Baseline/6 months/12 months: NYHA, QoL (MLHFQ), 6MWT, TTE, ECG, blood samples	MMP-2, TIMP-1	↑>10% 6MWT or if test not performed ↑>1 NYHA and survived/ no heart transplant at 12 months	25 (59.6%)
Truong *et al*[25]	Prospective observational	73	73	LVEF<35%, NYHA II–IV, QRS≥120 ms, OMT, HF decompensation <12 months	24 months (IQR 20.4–24.0)	Baseline: NYHA, ECG, TTE 1 month/3 months 6 months Clinical FU	Gal-3	Improvement HF clinical composite score[30] at 6 months	40 (54.7%)
Umar *et al*[10]	Prospective observational	64	64	LVEF<35%, NYHA III–IV, QRS>120ms	6 months	Baseline/6 months: NYHA, TTE; QoL (MLHFQ), 6MWT, blood samples	PINP, PIIINP, ICTP, proMMP-1, TIMP-1	↓≥10% LVESV at 6 months	46 (71.8%)
Garcia-Bolao *et al* [9]	Prospective observational	61	59	LVEF≤35%, NYHA III–IV, LBBB, QRS≥130 ms, OMT	12 months	Baseline/12 months: NYHA, QoL (MLWHFQ), 6MWT, TTE, ECG, blood samples	PICP, CITP, MMP-1, MMP-2, MMP-9, TIMP-1	↑≥10% 6 MWT and survival from cardiac mortality at 12 months	35 (59.3%)
Lopez-Andres *et al*[8]*	Substudy randomised control trial: CARE-HF	260 (CARE-HF cohort available)	132 (CRT-P only)	LVEF<35%, NYHA III–IV, QRS>150 ms or 120–149 ms with echocardiographic dyssynchrony, OMT	Substudy: 18 months; CARE-HF: 29.4 months (range, 18.0–44.7)	Substudy Baseline/3 months/18 months: TTE, blood samples	PINP, PIIINP, ICTP, MMP-1, Gal-3	Survival and LVEF more than 35% at 18 months	CRT-p (n=108): 72 (66.6%); OMT (n=117): 103 (88.0%), p=0.0001

*Median (IQR).
CITP, carboxy-terminal telopeptide of collagen type-I; CRT, cardiac resynchronisation therapy; CRT-p, CRT-pacemaker; ECM, extracellular cardiac matrix; Gal-3, galectin-3; HF, heart failure; ICTP, carboxy-terminal telopeptide of type I collagen; LVEF, left ventricular ejection fraction; LVESV, left ventricular end-systolic volume; LVESVi, left ventricular end-systolic volume indexed; 6MWT, six min walk test; MLHFQ, Minnesota living with heart failure questionnaire; MMP, metalloproteinases; NYHA, New York Heart Association; OMT, optimal medical therapy; PINP, N-terminal propeptides of type I procollagens; PIIINP, N-terminal propeptides of type III procollagens; QoL=quality of life; SR, sinus rhythm; TIMP, tissue inhibitors of MMPs; TTE, transthoracic echocardiogram.

Table 2 Baseline characteristics

Study ID	Age (years)	Male gender	CRT-d	Device upgrade	Ischaemic aetiology	Atrial fibrillation	Medication	LBBB	QRS (ms)	NYHA	6MWT (M)	LV
Dong *et al*[26]	68±9	37 (82.2%)	45 (100%)	Not reported	26 (57.8%)	Chronic AF excluded	ACEi/ARB 27 (60.0%), BB 41 (91.1%)	23 (53.3%)	>120	3.03±0.33	351±186	LVESVi 77±26 mL/m^2, LVEF 26%±5%
Tolosana *et al*[24]	66±8	35 (83.3%)	25 (59.5%)	Not reported	19 (45.2%)	8 (19%)	ACEi/ARB 33 (78.5%), BB 27 (64.3%), MRA 20 (47.6%)	Not reported	≥120	≥III=33 (78.5%) or II=9 (21.4%) → pacing indication	232±126	LVESV 162±63 mL, LVEDV 212±66 mL, LVEF 27%±7%
Truong *et al*[25]	68±12	61 (83.6%)	Yes	41 (56.2%)	39 (53.4%)	34 (46.5%)	ACEi/ARB 57 (78.1%), BB 64 (87.7%), MRA 16 (21.9%)	39 (53.4%)	168±27	2.9±0.4	Not done	LVESV 163±60 mL, LVEDV 226±73 mL, LVEF 27%±7%
Umar *et al*[10]	64±11	52 (81%)	Yes	Not reported	45 (70.3%)	Not reported	Not reported	Not reported	162±24	3.1±0.2	330±114	LVESV 172±69 mL, LVEDV 229±78 mL, LVEF 25%±8%
Garcia-Bolao *et al*[9]	69±4	40 (67.8%)	33 (55.9%)	Not reported	30 (50.8%)	11 (18.6%)	ACEi/ARB 59 (100%), BB 34 (57.6%), MRA 21 (35.6%)	51 (86.4%)	158±35	3.1±0.6	327±112	LVEF 25%±5%
Lopez-Andres *et al*[8]*	66 (59–71)	90 (68%)	0 (0%)	Excluded in CARE-HF	53 (40.2%)	AF excluded	ACEi/ARB 131 (99.2%), BB 88 (66.7%), MRA 73 (55.3%)	Not reported	160 (152–180)	3.0±0.2	Not done	(n=115) LVESV 206 mL (174–272), LVEDV 274 mL (233–355), LVEF 25% (21–29)

*Median (IQR).
ACEi, angiotensin converting enzyme inhibitor; AF, atrial fibrillation; ARB, angiotensin receptor blocker; BB, beta blocker; CRT-d, cardiac resynchronisation therapy defibrillator; CARE-HF, cardiac resynchronisation in heart failure; LBBB, left bundle branch block; LV, left ventricular; LVESV, left ventricular end-systolic volume; LVESVi, left ventricular end-systolic volume indexed; LVEDV, left ventricular end diastolic volume; 6MWT, six min walk test; MRA, mineralcorticoid receptor blocker; NYHA, New York Heart Association.

three studies were likely due to differences in response definitions and baseline characteristics. Garcia-Balao *et al*[9] utilised a clinical definition of response, whereas the other two studies used echocardiographic criteria.[8 10] All studies varied in duration of follow-up. Umar *et al*[10] had a higher proportion of men with ischaemic aetiology than the other studies. Lopez-Andres *et al*[8] excluded AF, whereas within the Garcia-Balao *et al*[9] cohort it was present in 18.6% of participants. Garcia-Balao *et al*[9] tested the predictive value of type I collagen turnover with the PICP:CITP ratio with a ratio ≥14.4 predicting response.

PIIINP

Variation was reported in trends of PIIINP levels at baseline. Dong *et al*[26] reported logarithmic figures making absolute figure comparison challenging. Geometric means could be calculated, but given small numbers of participants this was likely to underestimate the true mean.[26] Higher PIIINP levels in HF versus healthy controls (0.88±0.21 ug/L vs 0.71±0.14 ug/L, p=0.01) were observed.[26] Responders had significantly lower PIIINP baseline levels than non-responders (p=0.03).[26] Umar *et al*[10] demonstrated no difference in baseline levels between RvsNR. Lopez-Andres *et al*[8] reported similar baseline levels between CRT-p and OMT, but did observe PIIINP (>4.7 ug/L) in univariate analysis predicted cardiovascular outcomes (death or HF hospitalisation at 18 months) (OR 1.80, 95% CI 1.06 to 3.06, p=0.03).[8]

ICTP or CITP

Both ICTP and CITP were used to represent carboxyl-terminal peptides of type I collagen in three included studies. Umar *et al*[13] and Garcia-Balao *et al*[12] demonstrated similar baseline means for ICTP/CITP for the entire cohort. Neither identified independent predictors of CRT response.[9 10] Garcia-Bolao *et al*[9] identified that the PICP:CITP ratio strongly predicted response but was driven by PICP. Lopez-Andres *et al*[8] observed similar expression between CRT-p and OMT groups and showed no predictive value.

MMP-1, MMP-2 and MMP-9

There were variations in reported baseline concentrations for MMP-1. The mean for MMP-1 in Garcia-Bolao *et al*[9] was higher than median observed in CRT-p and OMT groups in Lopez-Andres *et al*,[8] though the data skew is unknown. Garcia-Bolao *et al*[9] examined the predictive value of MMP-1:TIMP-1, given their intrinsic regulatory role in collagen turnover,[5] but showed no statistical significance. Lopez-Andres *et al*[8] observed with a baseline MMP-1 ≤3 ug/L an adjusted threefold increased risk of CRT non-response and an increased risk of death or N-terminal *pro* B-type natriuretic peptide >1000 ng/L (OR 2.23, 95% CI 1.00 to 5.00, p=0.051/0.073 adjusted with/without renal function).[8] A precursor to MMP-1 called pro-MMP-1 (pro-MMP-1) was studied by Umar *et al*.[10] They observed no difference in baseline pro-MMP-1 expression between RvsNR.[10]

Table 3 Baseline ECM biomarkers concentrations between responders versus non-responders and their predictive value within included studies

ECM	Study ID	Baseline	Model	Predicting response
PINP	Umar *et al*[10]~	TC: 35.4±5.0 ug/l; R: 32.9±2.2 ug/L; NR: 41.9±4.3 ug/L, p=0.04	(Stepwise) multiple logistic regression‡	Univariate: OR 0.99, 95% CI 0.93 to1.00, p=0.05 Multivariable: OR 0.96, 95% CI 0.93 to 0.99, p=0.03
	Lopez-Andres *et al*[8]*	CRT-p: 33.0 ug/L (24.6–49.4); OMT: 33.1 ug/L (23.0–49.3), p=NS	(Stepwise) Multiple logistic regression†	No association with response
PICP	Garcia-Bolao *et al*[9]	TC: 74.3±29.9 ug/l; R: 85.6±29.4 ug/L; NR: 57.8±22.2 ug/L, p≤0.001	ROC:PICP:CITP	AUC 0.71, 95% CI 0.57 to 0.85; Cut-off 14.4, 95% CI 9.8 to 17.7; Sensitivity 63% (51–80); Specificity 70% (50–85); OR 2.07, 95% CI 0.98 to 4.39
PIIINP	Dong *et al*[26]≠	TC: 0.88±0.21 ug/l; R: 0.80±0.20 ug/L; NR: 0.96±0.19 ug/L, p=0.03	(Stepwise) multiple logistic regression‡	Univariate: OR 0.77, 95% CI 0.62 to 0.97, p=0.03 Multivariable: OR 0.20, 95% CI 0.03 to 1.17, p=0.07
	Umar *et al*[10]~	R: 4.59±0.24 ug/L; NR: <responders, p=NS	(Stepwise) multiple logistic regression‡	Univariate: OR 1.23, 95% CI 0.86 to 1.76, p=0.23 Multivariable: OR 1.35, 95% CI 0.94 to 1.93, p=0.1
	Lopez-Andres *et al*[8]*	CRT-p: 4.6 ug/L (3.8–6.8); OMT: 4.7 ug/L (3.8–6.5), p=NS	(Stepwise) multiple logistic regression†	No association with response
ICTP	Umar *et al*[10]~	TC: 3.1±0.8 ug/l; R: 3.5±0.6 ug/L; NR: 2.1±0.4 ug/L, p=ns	(Stepwise) multiple logistic regression‡	Univariate: OR 1.24, 95% CI 0.93 to 1.66, p=0.13 Multivariable: No association with response
	Lopez-Andres *et al*[8]*	CRT-p: 4.1 ug/L (2.6–6.0); OMT: 3.4 ug/L (2.7–5.0), p=NS	(Stepwise) multiple logistic regression†	No association with response
CITP	Garcia-Bolao *et al*[9]	TC: 5.1±2.5 ug/l; R: 4.90±2.5 ug/L; NR: 5.3±2.5 ug/L, p=0.51	ROC:PICP:CITP	AUC 0.71, 95% CI 0.57 to 0.85; Cut-off value 14.4, 95% CI 9.8 to 17.7; Sensitivity 63% (51–80); Specificity 70% (50–85); OR 2.07, 95% CI 0.98to 4.39
ProMMP-1	Umar *et al*[10]~	TC: 7.7±0.8 ug/l; R: 7.6±0.7 ug/L; NR: 8.0±1.1 ug/L, p=0.71	(Stepwise) multiple logistic regression‡	Univariate: OR 0.97, 95% CI 0.87 to 1.09, p=0.71 Multivariable: No association demonstrated
MMP-1	Garcia-Bolao *et al*[9]	TC: 8.9±11.4 ug/l; R: 7.3±10.5 ug/L; NR: 11.3±12.5 ug/L, p=0.17	ROC	Not performed as no difference Baseline MMP-1:TIMP-1 ratio: 0.005±0.001, R: 0.004±0.0007 versus NR: 0.0063±0.0008, p=0.297
	Lopez-Andres *et al*[8]*	CRT-p: 2.7 ug/L (2.1–3.5); OMT: 2.7 ug/L (2.0–3.9), p=NS	(Stepwise) multiple logistic regression†	Univariate ≤3 ug/L: OR 2.42, 95% CI 1.23 to 4.76, p=0.011 Multivariable ≤3 ug/L: OR 3.04, 95% CI 1.37 to 6.71, p<0.01
MMP-2	Tolosana *et al*[24]	TC:295±70 ug/L; R:258±56 ug/L; NR:325±116 ug/L, p=0.02	Cox Regression Model‡	Univariate: difference already noted (p=0.02) Multivariable: No association demonstrated
	Garcia-Bolao *et al*[9]	TC: 1434±401.5 ug/l; R: 1393.8±374.5 ug/L; NR: 1496.6±438.9 ug/L, p=0.36	ROC	Not performed as no difference demonstrated
MMP-9	Garcia-Bolao *et al*[9]	TC: 44.7±23.2 ug/l; R: 41.1±22.8 ug/L; NR: 49.9±23.3 ug/L, p=0.17	ROC	Not performed as no difference demonstrated
TIMP-1	Tolosana *et al*[24]	TC:242±61 ug/l; R:216±50 ug/L; NR:277±59 ug/L, p=0.001	Cox Regression‡; ROC	Multivariate: OR 0.97, 95% CI 0.96 to 0.99, p=0.005, ROC: ≥248 ug/L, Sensitivity 71%, Specificity 72%, OR 6.8, 95% CI 1.5 to 31
	Umar *et al*[10]~	TC: 120.3±8.2 ug/l; R: 124±5.2 ug/L; NR: 111±7.1 ug/L, p=0.16	(Stepwise) multiple logistic regression‡	Univariate: OR 1.01, 95% CI 0.99 to 1.03, p=0.16 Multivariable: No association demonstrated
	Garcia-Bolao *et al*[9]	TC: 488.9±249.5 ug/l; R: 437.5±136.5 ug/L; NR: 563.8±345.7 ug/L, p=0.135	ROC	Not performed as no difference. Baseline MMP-1:TIMP-1:TC: 0.005±0.001, R: 0.004±0.0007 versus NR: 0.0063±0.0008, p=0.297
Gal-3	Truong *et al*[25]*	TC: 18.1 ug/L (14.0–23.0), Positive result >25.9 ug/L	2×2 table; McNemar test	Peripheral: Sensitivity 15% (5–32), Specificity 80% (64–91), PPV: 38% (14–68), NPV: 53% (40–66)
	Lopez-Andres *et al*[8]*	CRTp: 25.7 ug/L (20.6–31.4); OMT: 25.1 ug/L (19.6–30.9), p=NS	(Stepwise) multiple logistic regression†	No association with response

*Median (IQR) given, data represented as CRT-p and OMT groups.
†Statistical model predicts 'non-response'.
‡Statistical model predicts 'response'.
~ mean±standard error.
≠biomarker mean±SD given in logarithmic form
AUC, area under the curve, CITP, carboxy-terminal telopeptide of collagen type-I; CRT-p, CRT-pacemaker; ECM, extracellular cardiac matrix; Gal-3, galectin-3; MMP, metalloproteinases; NPV, negative predictive value; NS=not significant; NR=non-responder; OMT, optimal medical therapy; PICP, carboxy-terminal propeptide of procollagen type I; PINP, N-terminal propeptides of type I procollagens; PIIINP, N-terminal propeptides of type III procollagens; PPV, positive predictive value; R, responder; ROC, receiver operator curve; TC, total cohort; TIMP, tissue inhibitors of MMPs.

Two studies reported cohort means for MMP-2 baseline concentration with large differences (table 3). Responders had lower MMP-2 baseline concentrations in both studies. Tolosana *et al*[24] reported a significant difference between RvsNR (p=0.02), whereas Garcia-Bolao *et al*[9] demonstrated no difference. The differences are not fully explained by study design, response definition or cohort characteristics as they showed similarities (tables 1 and 2). Variation in levels may be due to Tolosana *et al*[24] using plasma and Garcia-Bolao *et al*[9] using serum in their sandwich ELISAs. MMP-9 was reported by Garcia-Bolao *et al*,[9] who observed a trend towards lower baseline MMP-9 concentration for Responders. Baseline MMP-9 did not predict CRT response.[9]

TIMP-1

Tolosana *et al*[24] observed that responders had significantly lower concentrations at baseline of TIMP-1 than non-responders. Neither Umar *et al*[10] nor Garcia-Bolao *et al*[9] observed a significant difference in baseline TIMP-1 concentration between RvsNR. Higher peripheral TIMP-1 was identified as an independent predictor of non-response by Tolosana *et al*[24] in multivariable analysis; a concentration of ≥248 ug/L had a 71% sensitivity and 72% specificity for predicting non-response. However, Umar *et al*[10] did not identify TIMP-1 as a predictor. Garcia-Bolao *et al*[9] tested TIMP-1 in the MMP-1:TIMP-1 ratio and did not identify TIMP-1 as a significant predictor of RvsNR.

Gal-3

Lopez-Andres *et al*[8] reported higher baseline levels of Gal-3 than Truong *et al*,[25] due to different response definitions and variation in cohort characteristics. Lopez *et al*[8] used an echocardiographic definition at 18 months and Truong *et al*[25] utilised HF clinical composite score at 6 months. Truong *et al*[25] has higher ischaemic aetiology (53.4% vs 40.2%) and included patients with AF. Neither study reported baseline concentrations for RvsNR.[8 25] Truong *et al*[25] observed that peripheral baseline Gal-3 above a preset concentration (>25.9 ug/L) had low sensitivity and high specificity for predicting CRT response.

DISCUSSION

The ECM is a highly dynamic structure that is integral to myocardial structure and function which detrimentally remodels following cardiac injury leading to the altered turnover, replacing contractile tissue with collagen rich connective tissue and ultimately the development of myocardial fibrosis.[5] Myocardial fibrosis is characterised by adverse remodelling which contributes to systolic and diastolic HF.[5 28] PINP, PICP and PIIINP are released into the circulation during conversion and deposition of procollagen to collagen and are upregulated during myocardial fibrosis and associated with adverse HF outcomes.[5 7 15 28] Mechanistically, higher upregulation of collagen would challenge a CRT's ability to reverse remodel and for the patient to respond. Umar *et al*[10] supported this hypothesis observing significantly lower baseline PINP expression predicted echocardiographic response. Dong *et al*[26] did observe lower baseline PIIINP predicted echocardiographic response on univariate analysis, but not multivariable analysis. In contrast, Garcia-Balao *et al*[9] observed higher baseline expression of PICP in responders and PICP:CITP ratio (type I collagen turnover) of ≥14.4 had greater than twofold increased chance of predicting functional response, driven by PICP. Critically, echocardiographic and clinical/functional response criteria correlate poorly,[18] so could not be contrasted. Importantly, Lopez-Andres *et al*,[8] the largest study included in the review, did not observe upregulation of collagen synthesis predicting echocardiographic non-response, which does challenge the Umar *et al*[10] and Dong *et al*[26] observations; however, the cohort characteristics and study designs were different. The observations of collagen synthesis following CRT implantation conflict with each other. Umar *et al*[10] reported a significant increase in PINP and decrease in PIIINP expression in responders at 6 months; both would mechanistically be expected to be lower at follow-up. In contrast Garcia-Bolao *et al*[9] observed PICP levels decreased for responders and increased for non-responders at 1 year, which would be expected, but is based on a functional response definition. In contrast to collagen synthesis, degradation biomarkers (ICTP or CITP) did not predict CRT response.[8–10] Furthermore, no significant change in ICTP or CITP expression was observed at follow-up across all three studies.[8–10] Alteration in collagen synthesis rate is observed to be more powerful at predicting response than collagen degradation. Different patterns of collagen synthesis biomarkers predicting response have been observed; lower expression predicted LV reverse remodelling,[10 26] whereas higher rates predicted functional response.[9] The variation in these patterns is explained by the different response definitions and cohort characteristics. The study cohort for Umar *et al*[10] had a higher proportion of men and ischaemic cardiomyopathy than Garcia-Bolao *et al*.[9] The heterogeneities between these studies make drawing conclusions difficult. Lopez-Andres *et al*[8] also challenge any observations due to size of cohort and no prediction value to collagen turnover observed. Overall, collagen synthesis is observed to be important in predicting CRT response, especially LV reverse remodelling, with results replicated in two studies that lower rates predict LV reverse remodelling.[10 26]

MMP-1, MMP-2 and MMP-9 perform a critical role in myocardial collagen degradation and have been identified as being important prognostic markers in HF.[11 13 27] Predictive value for CRT non-response (death or LVEF ≤35% at 18 months) was only demonstrated in baseline MMP-1 expression ≤3 ug/l[8] supporting an observation by Jordan *et al*[11] that lower MMP-1 inferred worse HF prognosis. MMP-2 had large variations observed between the included studies,[8 24] but was not demonstrated to predict response. MMP-9 was only observed in one included study showing no predictive value[9];

however, recently Dini *et al*[13] demonstrated raised levels (>238 ng/mL) and predicted worse HF outcomes. MMP activity was not considered in any of these studies as a predictor but would be important to consider in the future. Current evidence suggests that MMPs, especially MMP-2 and MMP-9, have not yet had their potential fully evaluated.

TIMP-1 regulates the endogenous proteolytic MMP system involving discordant inhibition and in chronic inflammatory states stimulating collagen synthesis and myocardial fibrosis.[5 24] Tolosana *et al*[24] observed a significant baseline difference in RvsNR expression with lower TIMP-1 in responders. Tolosana *et al*[24] demonstrated that baseline TIMP-1 (≥248 ug/L) predicted CRT non-response. Trucco *et al*[29] in long-term follow-up of the same cohort demonstrated that the same threshold independently predicted mortality at 60±34 months (sensitivity 80% and specificity 71%). Tolosana *et al*[24] also demonstrated that statistically significant lower TIMP-1 is found in participants that do LV reverse remodel (LVESV reduction ≥10%). Umar *et al*[10] and Garcia-Bolao *et al*[9] observed no difference statistically at baseline. Variation between the reported literature in the magnitude of association of TIMP-1 exists; however, Tolosana *et al*[24] offers a well-designed prospective observational study which is powered giving strength to the conclusions drawn.

Gal-3 stimulates fibroblasts to release TIMPs and MMPs that regulate collagen turnover, resulting in myocardial fibrosis.[14] Elevated levels are independent predictors of adverse outcomes in HF.[14] Evaluation of Gal-3 as a predictor of response was limited, as RvsNR was not reported in either of the two studies.[8 25] Truong *et al*[25] demonstrated peripheral baseline Gal-3 ≥25.9 ug/L had specificity for predicting CRT response. Lopez-Andres *et al*[8] observed Gal-3 baseline expression ≥30 ng/L had nearly threefold increased risk of death or hospitalisation for worsening HF following CRT. Though not demonstrated to be a strong predictor, the evidence suggests that Gal-3 is a good biomarker for predicting poor outcomes in HF and needs further evaluation.

The greatest challenge for research into CRT response and one this review demonstrated is lack of an accepted response definition. Differing definitions rarely correlate,[18] which our review clearly demonstrates. Echocardiographic and clinical/functional definitions correlate very poorly and should never be compared or applied in a composite definition[18]; LV reverse remodelling should be considered separately.[18 30]

Study limitations

Heterogeneity among included studies was widespread despite a rigorous eligibility and screening criteria. The variations in study design, cohort characteristics and response definitions made pooling data in a meta-analysis impractical. CRT implantation techniques and indications have evolved over the last 15 years and offer another source of heterogeneity. Furthermore differences in laboratory techniques account for some variation among biomarker results. These limitations are particularly important to consider in future research studies.

CONCLUSIONS

Collagen synthesis biomarkers have shown the most potential, particularly PINP and PIIINP, but will require further study. MMP-2 and MMP-9 have no conclusive predictive value and need further investigation. Heterogeneity is the greatest challenge for research in this field and needs to be minimised in future studies. The most important initial step is for a universal response definition to be adopted and applied.

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